PB83-144766

Plans for Clinical and Epidemiologic Follow-up after Area-wide Chemical Contamination
Proceedings of an International Workshop Held at Washington, DC on March 17-19, 1980

(U.S.) National Research Council
Washington, DC

Prepared for

Environmental Protection Agency
Washington, DC

1982

U.S. Department of Commerce
National Technical Information Service
NTIS

PB83-144766

Plans for Clinical and Epidemiologic Follow-up After Area-Wide Chemical Contamination

Proceedings of an International Workshop

Committee on Response Strategies to Unusual Chemical Hazards

Board on Toxicology and Environmental Health Hazards

Assembly of Life Sciences

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

50272-101

REPORT DOCUMENTATION PAGE

1. REPORT NO.

2.

3. Recipient's Accession No. PB83 144766

4. Title and Subtitle
Plans for Clinical and Epidemiologic Follow-up After Area-wide Chemical Contamination. Proceedings of an International Workshop, Washington, DC, Mar. 17-19, 1980

5. Report Date 1982

6.

7. Author(s)
Committee on Response Strategies to Unusual Chemical Hazards

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
National Research Council
Board on Toxicology and Environmental Health Hazards
2101 Constitution Avenue NW
Washington, D.C. 20418

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C) EPA 68-02-3211
(G)

12. Sponsoring Organization Name and Address
Environmental Protection Agency; Centers for Disease Control

13. Type of Report & Period Covered
Final

14.

15. Supplementary Notes
Proceedings of an International Workshop, Washington, D.C. Mar. 17-19, 1980

16. Abstract (Limit: 200 words)

This workshop was an undertaking of the Committee on Response Strategies to Unusual Chemical Hazards and its counterpart Italian Committee. The Committee were established in 1979 as a result of the 1976 chemical explosion in Seveso, Italy to develop guildelines that might be used to implement a world-wide mechanism for guiding biomedical researchers at the scenes of accidents similar to that at Seveso, and to eveluate newer health data from the Seveso accident and the design of future studies.

The topic of the workshop, Plans for Clinical and Epidemiologic Follow-up After Area-wide Chemical Contamination, was approached from two points of view: first, by exploring a number of cases in which such widespread contamination occurred and which served as the basis for field studies; and second, by evaluating diseases and target organs that were identified as likely outcomes of chemical exposures. A synthesis of experiences and guiding principles for future investigations of similar exposures was provided by a panel of experts from the U.S. and Italy.

17. Document Analysis a. Descriptors
Environmental exposures, reproductive injury, epidemiologic monitoring

b. Identifiers/Open-Ended Terms TCDD, Kepone, DBCP, Methyl Mercury, Chlorinated Hydrocarbons, PBBs, Atomic Bomb Casualty Commision, Love Canal, Seveso, PCBs

c. COSATI Field/Group

18. Availability Statement
This report has been approved for public sale; its distribution is unlimited

19. Security Class (This Report)
Unclassified

20. Security Class (This Page)
Unclassified

21. No. of Pages
431

22. Price

(See ANSI-Z39.18)

See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

Plans for Clinical and Epidemiologic Follow-up After Area-Wide Chemical Contamination

Proceedings of an International Workshop

Washington, D.C.
March 17-19, 1980

Luigi Dardanoni, Co-Chairman
Robert W. Miller, Co-Chairman
Committee on Response Strategies to Unusual Chemical Hazards
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences
National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1982

i.a

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The National Research Council was established by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and of advising the federal government. The Council operates in accordance with general policies determined by the Academy under the authority of its Congressional charter of 1863, which establishes the Academy as a private, nonprofit, self-governing membership corporation. The Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in the conduct of their services to the government, the public, and the scientific and engineering communities. It is administered jointly by both Academies and the Institute of Medicine. The National Academy of Engineering and the Institute of Medicine were established in 1964 and 1970, respectively, under the charter of the National Academy of Sciences.

The work on which this publication is based was performed pursuant to Contract 68-02-3211 with the Environmental Protection Agency and Centers for Disease Control.

COMMITTEE ON RESPONSE STRATEGIES TO UNUSUAL CHEMICAL HAZARDS

Robert Miller, Chairman
Clinical Epidemiologic Branch
National Cancer Institute
Bethesda, Maryland

A.L. Burlingame
Mass Spectrometry Research Resource
University of California
Berkeley, California

Aaron B. Lerner
Department of Dermatology
Yale University
New Haven, Connecticut

John A. Moore
Research Resources Program
National Institute of ENvironmental
Health Sciences
Research Triangle Park, North Carolina

Sheldon D. Murphy
Department of Pharmacology
University of Texas
Houston, Texas

Robert A. Neal
Department of Biochemistry
Vanderbilt University
Nashville, Tennessee

Milos Novotny
Department of Chemistry
Indiana University
Bloomington, Indiana

Patrick O'Keefe
Division of Laboratories and Research
New York Sate Department of Health
Albany, New York

Alan Poland
Department of Oncology
McArdle Lab for Cancer Research
University of Wisconsin,
Madison, Wisconsin

Staff

Robert G. Tardiff Project Director	Frances Peter Editor	Jacqueline Prince Staff Assistant

BOARD ON TOXICOLOGY AND ENVIRONMENTAL HEALTH HAZARDS

Ronald W. Estabrook, *Chairman*
Department of Biochemistry
University of Texas Medical School
Dallas, Texas

Philip Landrigan, *Vice-Chairman*
National Institute for
Safety and Health
Cincinnati, Ohio

Edward Bresnick
Department of Biochemistry
University of Vermont
Burlington, Vermont

Theodore Cairns
Greenville, Delaware

Victor Cohn
Department of Pharmacology
George Washington University
Medical Center
Washington, D.C.

A. Myrick Freeman
Department of Economics
Bowdoin College
Brunswick, Maine

Ronald W. Hart
National Center for Toxicological
Research
Jefferson, Arkansas

Michael Lieberman
Department of Pathology
Washington University
St. Louis, Missouri

Richard Merrill
School of Law
University of Virginia
Charlottesville, Virginia

Robert A. Neal
Chemial Industry Institute
of Toxicology
Research Triangle Park, North Carolina

Ian Nisbet
Clement Associates
Washington, D.C.

John Peters
Department of Family and
Preventive Medicine
University of Southern Cal
Los Angeles, California

Liane Russell
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Charles R. Schuster,Jr.
Department of Psychiatry
University of Chicago
Chicago, Illinois

Ex Officio Members

James F. Crow
Genetics Department
University of Wisconsin
Madison, Wisconsin

Roger McClellan
Lovelace Biomedical and
Environmental Research Inst
Albuquerque, New Mexico

Daniel Menzel
Department of Pharmacology
Duke University
College Park, Maryland

Robert Menzer
Department of Entomology
University of Maryland
College Park, Maryland

Robert Miller
National Cancer Institute
Bethesda, Maryland

Sheldon Murphy
Department of Pharmacology
University of Texas
Houston, Texas

Norton Nelson
Institute of Environmental Medicine
New York University Medical Center
New York, New York

James Whittenberger
School of Public Health
Harvard University
Boston, Massachusetts

Staff

Robert G. Tardiff
Executive Director
Board on Toxicology and Environmental
  Health Hazards
National Research Council

Gordon Newell
Associate Executive Director
Board on Toxicology and Environmental
  Health Hazards
National Research Council

Jacqueline Prince
Staff Assistant
Board on Toxicology and Environmental
  Heath Hazards
National Research Council

TABLE OF CONTENTS

Page

PREFACE xi

INTRODUCTION
Francesco Pocchiari....................................1

CASE STUDIES OF SELECTED AREA-WIDE ENVIRONMENTAL EXPOSURES

TCDD (Italy)
Luigi Dardanoni and Gaetano Fara........................3

Yusho (Japan)
Robert W. Miller.......................................34

Treatment of Chlordecone (Kepone) Poisoning with Cholestyramine
Philip S. Guzelian.....................................40

DBCP
Donald Whorton...60

Increased Lead Absorption with Anemia and and Slowed Nerve Conduction in Children Near a Lead Smelter
Philip J. Landrigan....................................75

Methyl Mercury (Japan)
Robert W. Miller.......................................86

Chlorinated Hydrocarbons
David Axelrod..90

Cohort Study of Michigan Residents Exposed to Polybrominated Biphenyls: Epidemiologic and Immunologic Findings
Philip J. Landrigan...................................100

Atomic Bomb Casualty Commission
Gilbert W. Beebe......................................114

Preceding page blank

ADVERSE EFFECTS ON TARGET SITES AND STUDIES OF TOXICITY

Reproductive Injury: Love Canal
David Axelrod....126

Reproductive Injury: General Considerations
Robert W. Miller....145

Reproductive Injury: General Considerations
Helga Rehder....154

Birth Defects Register in Seveso: A TTCDD-Polluted Area
E. Marni, et. al....174

Carcinogenic Effects of Chemical and Physical Agents: Human Observations
Clark W. Heath, Jr....195

Experimental Studies on Carcinogenic Effects of TCDD
Giuseppe Della Porta, Maria I. Culnaghi, Tommaso A. Dragani....206

Adverse Neurologic Effects
Alan M. Goldberg....217

Exposure to TCDD: Immunologic Effects
Girolamo G. Sirchia, et. al....234

Somatic Cell Mutations
Arthur D. Bloom....267

Cytogenetic Investigations of the Seveso Population Exposed to TCDD
L. De Carli, et al....292

Cytochrome P-450 Induction by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, Polychlorinated Biphenyls, and Polybrominated Biphenyls
Robert Neal....320

Hepatic Toxicity of TCDD
Silvio Garattini....335

In Vivo DNA Damaging Activity, In Vivo Covalent DNA Binding and Bacterial Mutagenicity as Related Quantitatively to Carcinogenic Potency
S. Parodi, M. Taningher, L. Santi......................364

Liver Injury
Sheldon D. Murphy......................................366

PANEL ON EPIDEMIOLOGIC APPROACHES TO MEASUREMENT AND ASSESSMENT OF EXPOSURES 385

SUMMARY AND CONCLUSIONS......................................406

APPENDIX 1: Epidemiologic Monitoring in an Episode of Environmental Chemical Pollution: Problems and Programs in the Seveso Experience
Leonardo Santi...409

APPENDIX 2: Program..413

PREFACE

In 1977, the National Academy of Sciences-National Research Council (NAS-NRC) was invited by the Italian government to join in a collaborative effort to investigate the effects of area-wide chemical contamination at Seveso, Italy. The contamination was the result of an explosion of a reaction vessel containing highly toxic 2,3,7,8-tetra-chloro-dibenzo-p-dioxin (TCDD and commonly known as "dioxin"), which produced a cloud of chemical that was carried southward by the wind, exposing humans, animals, and plant life for several kilometers. The NAS-NRC sent a team of American scientists to visit Italy and to determine with Italian officials the needs and opportunities for cooperative study. The NAS-NRC team recommended the development of a continuing relationship of U.S. and Italian scientists for the purposes of exchanging scientific and technical information, fostering the conduct of complementary research, organizing workshops and conferences to examine the impacts on health and the environment, and assisting in the coordination of exchange of scientists engaged in the analysis of this accident.

The NAS-NRC formally structured its involvement in this collaborative venture by establishing the Committee for Binational Cooperative Study of Exposure to TCDD which was later renamed the Committee on Response Strategies to Unusual Chemical Hazards. The Committee's terms of reference were two-fold: first, the development of guidelines that might be used to implement a worldwide mechanism for guiding biomedical researchers at the scene of accidents similar to that at Seveso (thereby ensuring the most comprehensive collection of scientific information in a timely manner); and second, the evaluation--in cooperation with Italian counterpart scientists--of newer health data from the Seveso accident and the design of future studies.

One of the Committee's first undertakings was a workshop, held in March 1980, on Plans for Clinical and Epidemiological Follow-up after Area-wide Chemical Contamination. These Proceedings are the product of that workshop. The topic of the workshop was approached from two points of view: first, by exploring a number of cases in which such widespread contamination occurred and which served as the basis for field studies; and second, by evaluating diseases and target organs that were identified as likely outcomes of chemical exposures. A synthesis of experiences and guiding principles for future investigations of similar exposures was provided by a panel of experts from the U.S. and Italy.

Between the time that the workshop was held and the proceedings were completed, the Committee has been aware of several publications on this topic and wishes to bring to the attention of the reader the following: Guidelines for Studies of Human Populations Exposed to Mutagenic and Reproductive Hazards (Proceedings of a conference held January 26-27, 1981, edited by Arthur D. Bloom, published by March of Dimes Birth Defects Foundation) and Chemical Radiation Hazards to Children (Proceedings of the 84th Ross Conference on Pediatric Research, Columbus, OH, in press). It is the Committee's hope that all of these resources will provide useful guidance to the difficult investigations of adverse effects to human health that may be associated with chemical exposures of individuals of broad geographic distribution.

INTERNATIONAL WORKSHOP ON PLANS FOR CLINICAL AND EPIDEMIOLOGIC FOLLOW-UP AFTER AREA-WIDE CHEMICAL CONTAMINATION

Introduction

Francesco Pocchiari

This International Workshop on "Plans for Clinical and Epidemiological Follow-up after Area-wide Chemical Contamination" is a collaborative effort of the Lombardy Region of Italy, the Istituto Superiore di Sanita in Rome, and the U.S. National Academy of Sciences, to deal with the long-term medical followup of the population exposed to dioxin at Seveso, and to explore the implications of this event toward similar, areawide chemical incidents. This meeting should help synthesize comprehensive epidemiologic approaches and develop plans for investigating widespread exposures and their impacts on human health for use in other present and future chemical accidents.

A number of problems have to be solved in order to define a medium-term strategy to both protect the population and to decontaminate the environment. In many respects, the Seveso incident is representative of the difficulties inherent in dealing with any accidental chemical contamination.

A major goal, in any such event, is the clinical and epidemiologic followup of the exposed population. But at Seveso, this undertaking was confounded by several factors:

1. Difficulty in identifying the actual "hit" area. TCDD distribution in soil was very uneven, with wide differences even between levels at nearby sites.

2. Constraints relative to the sampling and extraction techniques prevented extensive and rapid monitoring of contamination.

3. Lack of fully adequate and rapid analytic methods to assay TCDD in some materials, such as atmospheric particles, vegetation, and animal tissues resulted in delayed and uncertain estimates of the area actually exposed.

4. Overlapping responsibilities of national, regional, and municipal authorities required coordination.

An impressive amount of work was required to overcome all of the difficulties and, thus, to derive an exact estimate of the TCDD levels in the affected territory, to assess the persistence of TCDD in soil, and to predict with reliability the accident's effects on the health of the exposed population.

TCDD (Italy)

by Luigi Dardanoni and Gaetano Fara[1]

On July 10, 1976, a reactor exploded in Seveso, north of Milan, sending a cloud of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) over a large area north of Milan. Plants and animals in the vicinity of the plant were affected, as were humans exposed to the cloud. Levels of exposure were classified according to three geographic regions near the plant, and attempts were made to assess the risk to the residents of these areas, some of whom were evacuated. Techniques included assessment of soil samples from the various areas for TCDD contamination and, later, for TCDD persistence; a questionnaire survey of residents to determine their exposure to the cloud and to contaminated animals; pathologic studies of animals that spontaneously died in the area following the accident; autopsy reports of syndromes exhibited by those animals; and an examination of the general distribution of mortality among various species of animals. These data, along with the incidence of chloracne in the exposed population, were correlated with the territorial distribution of the TCDD derived from the soil samples.

This report summarizes the data on the sources of contamination by and the degree of population exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) contamination in Seveso, Italy. It focuses on TCDD distribution in different environmental areas within the Seveso region and on the degree of human exposure, based on an evaluation of anamnestic data provided by area residents, deaths of animals, and human morbidity. Most of the data presented here have already been published elsewhere.

Industria Chimica Meda Societa Anonoma (ICMESA), a chemical and pharmaceutical company, was established in Seveso in 1945. It began producing 2,4,5-trichlorophenol (2,4,5-T) in 1969. On July 10, 1976, a fast-spreading reaction raised the internal temperature of the reactor to far above 200°C (perhaps to 400°C), and the pressure increased up to the critical point of the valve. As a result, TCDD concentration

---

[1] Institute of Hygiene, University of Palmero Medical School (L. Dardanoni) and Institute of Hygiene, University of Milan Medical School (G. Fara).

in the final product was also raised beyond measure. When the protection disk blew up, the fluid mixture burst into the open air, propelled by the built-up pressure. The visible part of the cloud rose some 50 meters and subsequently fell back to the ground. The wind caused the TCDD to spread over a wide area.

Leaves of plants, courtyard animals, and birds near the ICMESA factory were severely affected. Many animals died within a few days of the accident. At the same time, humans exposed to the toxic alkaline cloud began to develop dermal lesions (Pocchiari _et al_., 1979).

A map approximating the contaminated area (Figure 1) was drawn based on airstream pattern at blow-out time, preliminary analytic findings, and information on the sites of toxic and pathologic events, which was obtained through work performed by university and county laboratories coordinated by the Istituto Superiore di Sanita. As a first step, on July 26, 1976, Italian authorities evacuated 179 people from a 15-hectare area immediately southeast of the plant. A few days later, further analysis of the TCDD content of soil and vegetation samples prompted authorities to evacuate all the inhabitants (733 people) from a wider area (Zone A, approximately 110 hectares), extending about 2 km southeast of the ICMESA plant. Moreover, inhabitants of the surrounding area (Zones B and R) were subjected to a number of public health regulations. Residents were prohibited from farming, consuming local agricultural products, and keeping poultry and other animals.

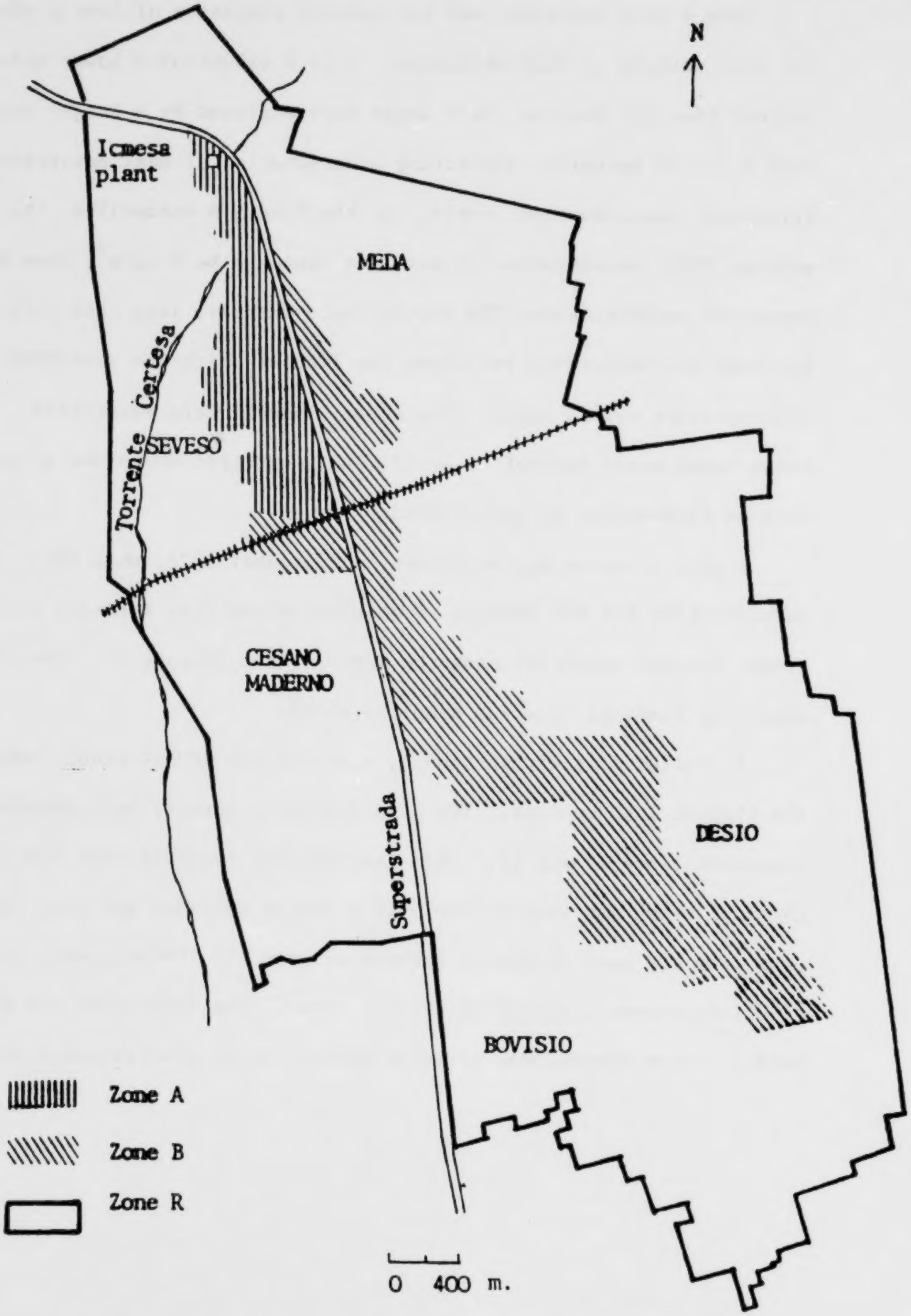

N
Icmesa plant
MEDA
Torrente Certesa
SEVESO
CESANO MADERNO
Superstrada
DESIO
BOVISIO
Zone A
Zone B
Zone R
0 400 m.

FIGURE 1. Schematic map of Seveso area by pollution zones. From Regione Lombardia, Ufficio Speciale, 1976.

Zone B (270 hectares) was the natural extension of Zone A along the main pathway of TCDD diffusion. Zone B exhibited a lower dioxin content than did Zone A. Both zones were enclosed by a larger territory, Zone R (1,430 hectares) exhibiting undetectable, or near detection-threshold, contamination levels. At the Zone B-R borderline, the average TCDD concentration in soil was found to be 5 $\mu g/m^2$; Zone R contained nondetectable TCDD levels (more exactly, less than 0.75 $\mu g/m^2$. The Zone A-B borderline ran along the line at which the mean TCDD concentration was 50 $\mu g/m^2$. The actual borders were eventually established where natural or artificial geographic divisions already existed (DiDomenico et al., 1980a).

A more accurate map was drawn in September 1976, when the investigators had the results of sampling along five straight reference lines, fanning southward from the ICMESA plant (Figure 2). The figure shows the findings from 108 sampling sites.

A limited area within Zone A, close to the ICMESA plant, exhibited the highest TCDD content. The main diffusion pathway fell between reference lines II and IV. This distribution suggests that most of the chemical cloud was wind-driven over a narrow corridor and that, moving away from the main diffusion pathway or from the ICMESA plant, a gradual dilution process occurred within the cloud. The dispersion was due both to three dimensional airborne motion and to gravitational effects.

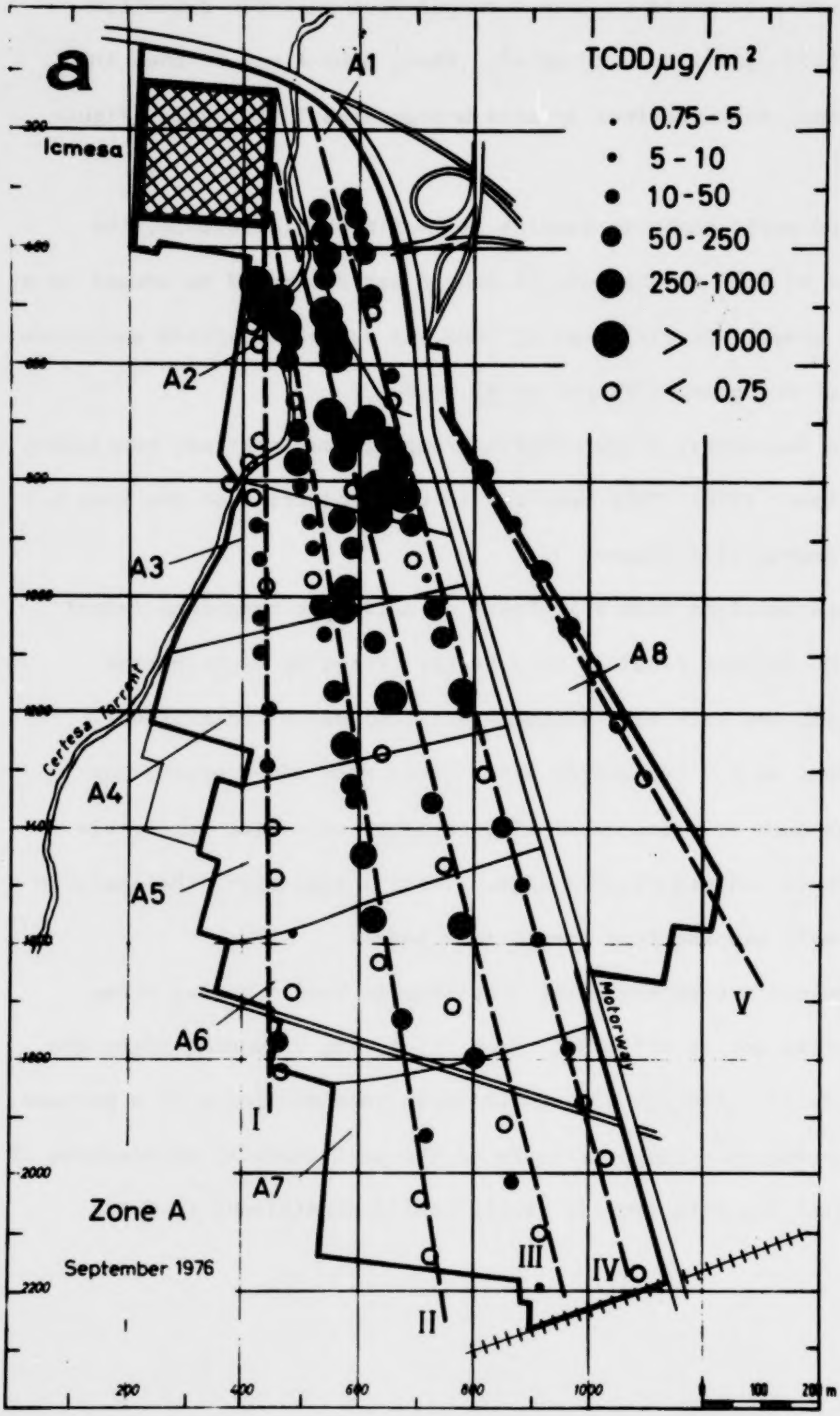
TCDD levels detected in Zone A ranged from analytic detection threshold (0.75 $\mu g/m^2$) to $\approx$ 20 mg/m^2. Thus, Zone A was divided into eight subzones, characterized by more homogeneous TCDD levels (Figure 2).

Based on early analytic results and various calculations, the total amount of TCDD in the soil of Zone A was estimated to amount to a few hundred grams. By far, most of TCDD was deposited within an 0.6-km area south of the plant (Bisanti et al., 1980).

By late September, a new sampling campaign was started, continuing through December 1976. This resulted in the production of the Zone A map dated January 1977 (Figure 3).

The data obtained from soil tests on different occasions reveal that the TCDD fallout resulted in a highly irregular distribution pattern, which has been only slightly modified since the accident. Concentrations in any two nearby sites (less than 100 m apart) may differ by as much as a factor of 100, thereby conferring negligible significance to individual site data. Table 1 summarizes the analytic results of soil samples from the various zones.

TCDD levels determined at 44 locations in Zone A during three surveys carried out at different times (1, 5, and 17 months after the ICMESA accident), although not specifically intended for such a purpose, were used to derive a rough estimate of the environmental persistence of TCDD in soil. The data provide statistically significant ($p<0.01$)



a
TCDD µg/m²
• 0.75 - 5
• 5 - 10
● 10 - 50
● 50 - 250
● 250 - 1000
● > 1000
○ < 0.75
Icmesa
A1
A2
A3
A4
A5
A6
A7
A8
Certesa Torrent
Motorway
I
II
III
IV
V
Zone A
September 1976
1400
1600
1800
2000
2200
200
400
600
800
1000
0
100
200 m

FIGURE 2. September 1976 map of Zone A. Original polar coordinates are labeled with Roman numerals I-V. From DiDomenico *et al.*, 1980a.

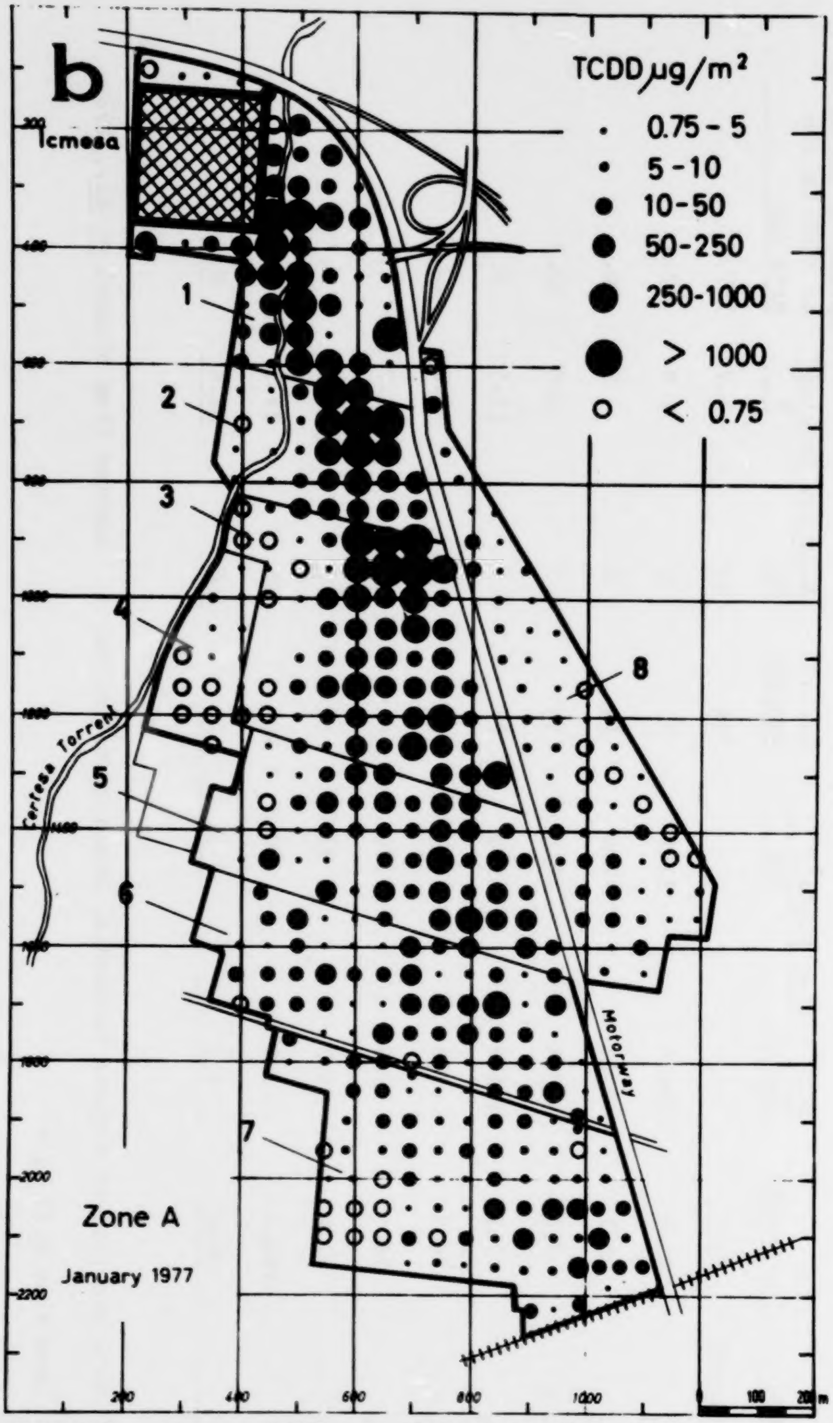

b
Icmesa
TCDD µg/m²
0.75 - 5
5 - 10
10-50
50-250
250-1000
> 1000
< 0.75
1
2
3
4
5
6
7
8
Certesa Torrent
Motorway
Zone A
January 1977
200
400
600
800
1000
100
200 m
-2000
-2200

FIGURE 3. January 1977 map of Zone A. From Di Domenico et al., 1980a.

TABLE 1

Distribution of TCDD Contamination in the A,B, and R Areas on the Basis of Gas Chromatographic-Mass Spectrometric Analysis of Soil Samples[a]

Zone	Size (hectares)	TCDD values ($\mu g/m^2$)		No. of Samples	Negative Samples[b]		Estimated Amount of TCDD in the Area (g)
		Average	Top		No.	%	
A total	80.3			306	12	3.9	147.5
A_1	10.7	580.4	5447	51	1	1.9	62.1
A_2	5.1	421.1	1700	19	0	0	26.5
A_3	9.2	350.5	2015	34	3	8.8	32.2
A_4	7.2	134.9	902	26	3	11.5	9.7
A_5	16.3	62.8	427	50	2	4.0	10.02
A_6	14.0	29.9	270	61	2	3.2	4.1
A_7	17.8	15.5	91.7	65	1	1.5	2.7
B	269.4	3.0	43.8	106	26	24.5	8.0
R	1,430.0[c]	0.9	9.7	449	308	68.6	8.5

[a] Data provided by Regione Lombardia, Piano Operativo no. 1. Adapted from Bisanti *et al.*, 1980.

[b] Less than 0.75 $\mu g/m^2$

[c] only 950 hectares mapped.

evidence that the geometric mean of TCDD levels dropped to about one-half in the unworked soil of Zone A during the first 5 months after the accident. Following this period, no further decreases in TCDD levels were detected. One month after the accident, TCDD half-life was approximately 1 year. Seventeen months after the accident, it was estimated to be greater than 10 years (Di Domenico et al., 1980b).

Vertical distribution of TCDD dropped sharply in the top 8 cm of soil, regardless of sampling site location or TCDD concentration. TCDD levels varied much less in the 8-24 cm range. This trend is illustrated in Figure 4, where the vertical distribution (expressed as a percentage of total TCDD recovered) observed at site A_{11} (a spot just south of the ICMESA plant in Zone A_1) is shown as a typical example. TCDD concentrations at depths greater than 8 cm were generally less than those detected in the upper layer at the site by at least one order of magnitude.

TCDD vertical distribution was also investigated in the top 2-cm soil layer. As Figure 5 shows, the highest TCDD levels were not found in the topmost soil layer (0.5 cm), but very often in the second (0.5-1.0 cm) or third (1.0-1.5 cm) strata (Di Domenico et al., 1980c).

This, and the findings of other investigators of the vertical distribution of TCDD in soil at various intervals following the accident, are consistent with the hypothesis that the TCDD abatement observed in 1976 at least partially resulted from photodegradation in the topmost layer, where most of the TCDD was exposed to direct sunlight.

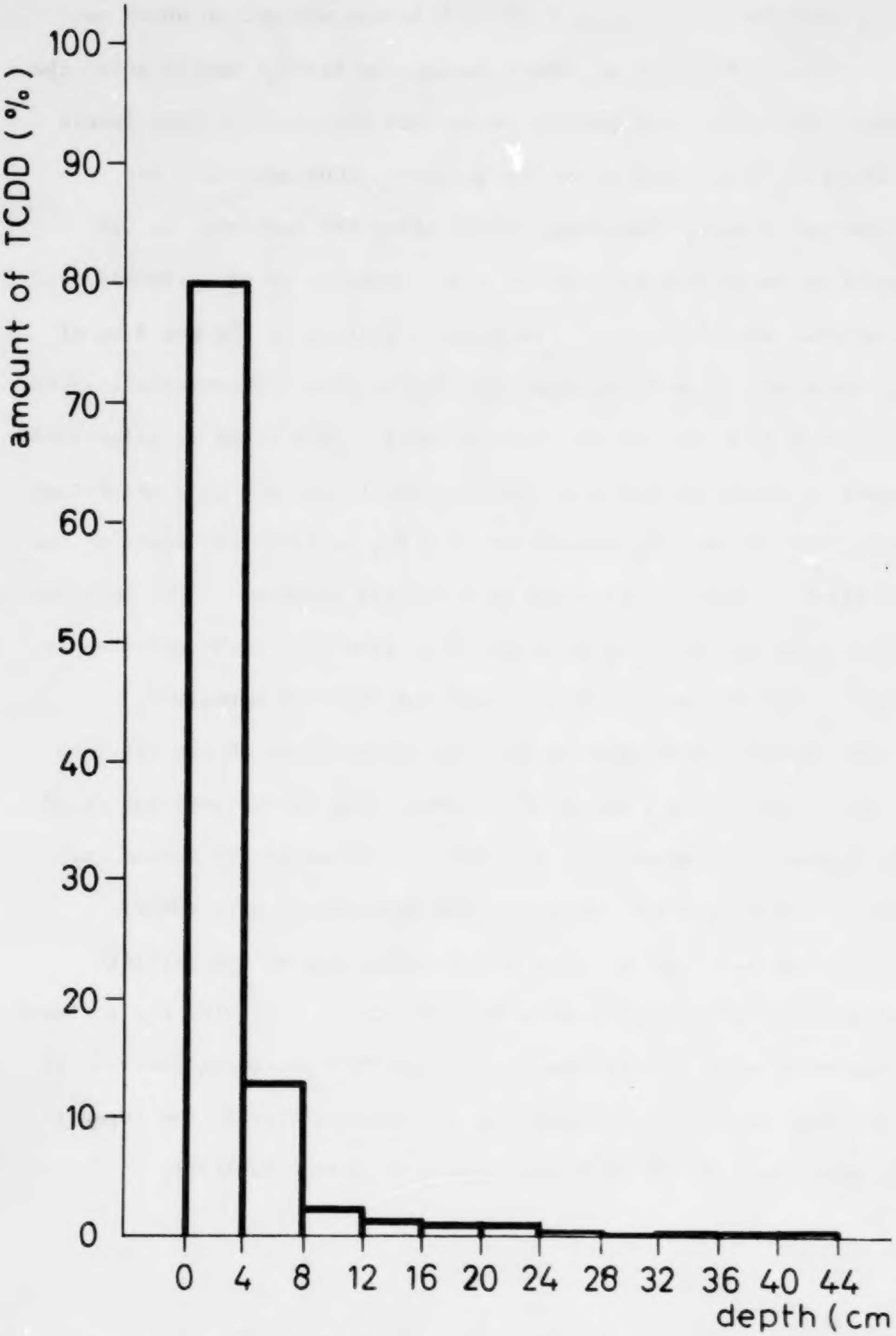


FIGURE 4. Site A_{11}: Vertical distribution of TCDD in soil, expressed as a percentage of total TCDD detected. Sampling was performed on September 27, 1976. From DiDomenico et al., 1980c.

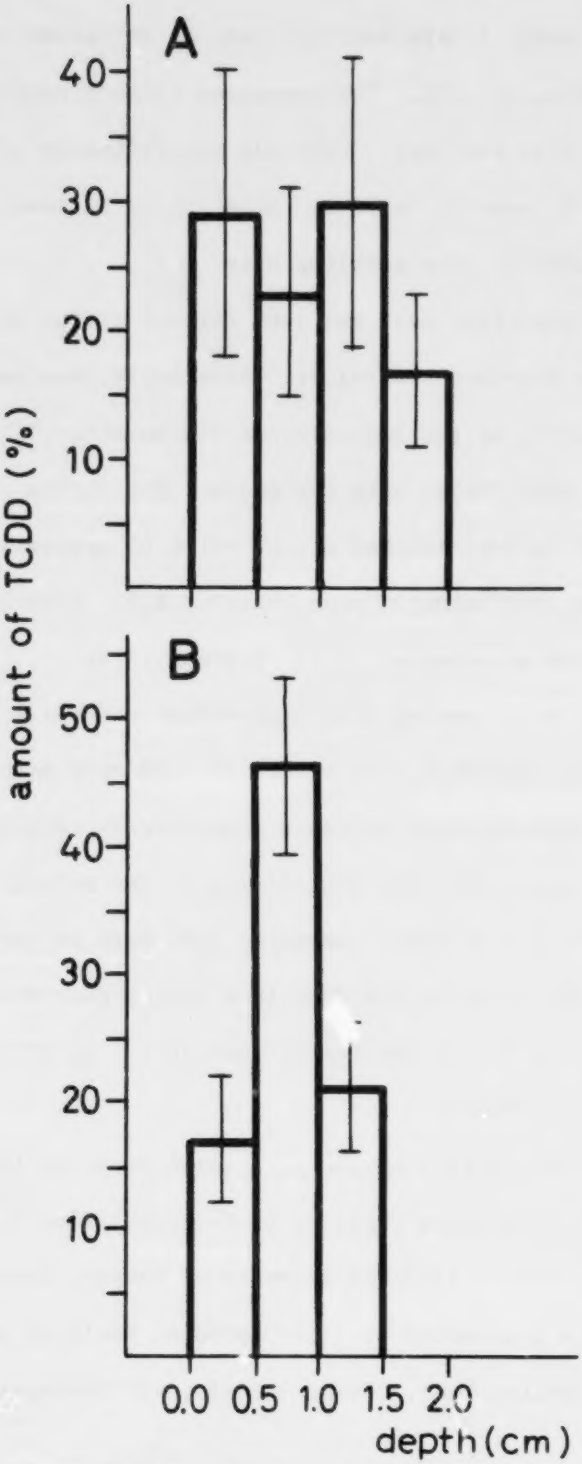


FIGURE 5. Vertical distribution of TCDD in the very top soil layer of Zone A (above) and Zone B (below). Each column represents the average of the five assays available for Zone A and of the 12 assays available for Zone B. From DiDomenico et al., 1980c.

Air particles were sampled with dustfall jars at different locations between July 1977 and February 1978. Two cosamples (liquid phase and sediment) were obtained from each jar. TCDD was identified in 15 of 19 sediment specimens from Subzone A_1, site 2. Seasonal variations in dust deposits were observed at this sampling site.

TCDD levels in the deposited dust and TCDD fallout at the site were calculated from the previous two values. Apparently, maximum dust and TCDD fallout occurs at the beginning of the summer. TCDD levels in settling dust also change with the season, but follow a different pattern. TCDD fallout reached a peak value of approximately 0.2 $ng/m^2/day$ in the most contaminated area (Subzone A_1). TCDD levels were occasionally recorded at other sites in 1978 and 1979.

Suspended particles were sampled with high-volume devices at five locations for 24 hours in June 1977. No traces of TCDD were detected; however, some TCDD was observed when extracts from several 24-hour samples were pooled and analyzed. The sensitivity of the method used was equal to 0.2 ng of TCDD/g of dust. Assuming that dust in the air is 0.14 mg/m^3 and the TCDD level in the dust is 1 ppb, a constantly exposed individual thus could have inhaled as much as 1.4 pg of TCDD daily (Di Domenico _et_ _al_., 1980d).

Table 2 shows the distribution of the population involved in the accident, by zone. Zone A includes segments of the population of two cities, Seveso and Meda; Zone B includes segments of Seveso, Cesano, and Desio; Zone R, with a population of 31,800 people, includes parts of six cities (Seveso, Cesano, Meda, Desio, Bovisio, and Barlassina).

TABLE 2

Distribution of Inhabitants (No. and %) by City[a] and Pollution Zone[b]

	No. and (%) Inhabitants					
Cities	Total	Zone A	Zone B	Zone R	Zone A+B+R	Zone non A+B+R
Seveso	16,975	668 (3.93)	628 (3.69)	7,945 (46.79)	9,241 (54.45)	7,734 (45.55)
Cesano M.	33,799	--	2,736 (8.09)	14,991 (44.35)	17,727 (52.44)	16,072 (47.56)
Total 1	50,774	668 (1.31)	3,364 (6.62)	22,936 (45.17)	26,958 (53.11)	23,806 (46.89)
Meda	19,571	62 (0.31)	--	4,017 (20.52)	4,079 (20.83)	15,492 (79.17)
Desio	33,011	--	1,373 (4.15)	4,608 (13.95)	5,981 (18.10)	27,030 (81.90)
Total 2	52,582	62 (0.11)	1,373 (2.61)	8,625 (16.40)	10,060 (19.14)	42,522 (80.86)
Bovisio M.	11,225	--	--	167 (1.48)	167 (1.48)	11,058 (98.52)
Barlassina	5,656	--	--	72 (1.28)	72 (1.28)	5,584 (98.72)
Seregno	36,838	--	--	--	--	36,838 (100)
Lentate	13,037	--	--	--	--	13,037 (100)

Table 2 (continued)

Cities	Total	Zone A	Zone B	Zone R	Zone A+B+R	Zone non A+B+R
	No. and % Inhabitants					
Varedo	11,841	--	--	--	--	11,841 (100)
Nova M.	19,467	--	--	--	--	19,467 (100)
Muggió	18,690	--	--	--	--	18,690 (100)
Total 3	116,754	--	--	239 (0.20)	239 (0.10)	116,515 (99.80)
Total 1+2+3	220,110	730 (0.33)	4,737 (2.15)	31,800 (14.47)	37,257 17.8	182,843 (82.06)

[a] The six cities with territory partially classified in one or more of the pollution zones (A,B,R) are members, with other cities, of three health administrative departments (Consorzi Sanitari di Zona or C.S.Z). Zone "non A+B+R", in the last column, included five cities that belong to the three C.S.Z. departments, but are completely outside the pollution zones (e.g., Seregno, Lentate, etc.).

[b] Adapted from Bisanti et al., 1980.

As illustrated in Table 2, the 11 cities can be divided into three groups. In the first group, Seveso and Cesano, more than 50% of the population was included in the contaminated area (A + B + R); in the second (Meda and Desio), 20% of the population was involved; and in the third group (seven cities) only 0.2% of the people were involved.

The health surveillance plan, as described by Fara (1976a), included the following:

1. clinical followup of each individual living in the polluted area, primarily for humanitarian reasons and at the request of the population, rather than for epidemiologic purposes;
2. longitudinal control of groups at higher risk (residents of Zone A, children of Zone B, ICMESA workers, residents of all zones who had suffered acute skin lesions or chloracne);
3. special projects, including searching for damages to reproductive, neurological, and immunologic systems, and studies of neoplastic and chromosomal effects; and
4. surveillance of general health indicators. Exposure data have been gathered through individual questionnaires to ascertain the following:

- exposure (E), i.e., presence in the polluted area on July 10, 1976;
- permanence (P), i.e., presence in the area from July 10 to August 10, 1976;

- death of poultry and domestic animals (A) due to suspected intoxication;
- eating of their own or neighbors' vegetables and farm products (C); and
- working in the open air (handling of agricultural products, working in the streets) or playing outdoors (for children) (G).

Information on exposure risk of any one person is translated into a configuration of the indicators P, E, A, C, and G, classified as follows and as shown in Table 3;

1. Absent or improbable risk: All risk indicators (replies) on the questionnaire are "most favorable" to the subject.
2. Uncertain risk: Information for one or more risk indicators is lacking. What data are available from the questionnaire can be considered "favorable" to the subject.
3. Definite risk: Information on the questionnaire for at least one of the risk indicators is "unfavorable" to the subject.

An attempt was made to correlate the exposure indicators to the incidence of chloracne by comparing the frequency of maximal risk configurations of 163 children with chloracne to 51 matched youngsters without the disease. As Table 4 shows, both diseased and nondiseased children showed similar exposure indicators.

Acute cutaneous lesions in humans, due to the toxic alkaline cloud, might also be considered a possible indicator of pollution. As Table 5 shows, the acute skin morbidity rate was higher in residents of Zone A than in residents of Zone B, but Zone R residents were not always less affected than were those in Zone B (Fara, 1976b). A similar incidence

TABLE 3

Classification of Risk for Individuals Exposed to TCDD[a]

Type of risk	Risk Associated with Factors for Each Individual[b]
Absent or improbable	$\overline{P}$ $\overline{E}$ $\overline{A}$ $\overline{C}$ $\overline{G}$
Uncertain	(P) E A C G
	$\overline{P}$ $\overline{E}$ $\overline{A}$ $\overline{C}$ $(\overline{G})$
	(P) E) $\overline{A}$ $\overline{C}$ $\overline{G}$
	$\overline{P}$ $\overline{E}$ $\overline{A}$ (C)(G)
	(P)(E)(A) $\overline{C}$ $\overline{G}$
	$\overline{P}$ $\overline{E}$ (A)(C)(G)
	(P)(E)(A)(C) $\overline{G}$
	$\overline{P}$ (E)(A)(C)(G)
	(P)(E)(A)(C)(G)
Certain	P $\overline{E}$ $\overline{A}$ $\overline{C}$ $\overline{G}$
	$\overline{P}$ E $\overline{A}$ $\overline{C}$ $\overline{G}$
	P E A C (G)
	P E A C G

---

[a] From Caramaschi et al., 1980.

[b] ( ) = Uncertain risk.

$\overline{X}$ = Risk not present.

TABLE 4

Frequency Distributions[a] for Subjects Under 15 Years of Age With and Without Chloracne[b]

Reported Risk	Selected Patterns[c]	Subjects with Chloracne No.	Subjects with Chloracne %	Subjects without Chloracne, Zone A No.	Subjects without Chloracne, Zone A %
None	$\overline{P}\ \overline{E}\ \overline{A}\ \overline{C}\ \overline{G}$	–	–	–	–
Uncertain	$\overline{P}\ \overline{E}\ (A)\ \overline{C}\ \overline{G}$	–	–	–	–
	$(P)\ (E)\ (A)\ (C)\ (G)$	9	16	–	–
Definite	$\overline{P}\ \overline{E}\ \overline{A}\ \overline{C}\ G$	–	–	–	–
	$\overline{P}\ \overline{E}\ (A)\ \overline{C}\ G$	–	–	–	–
	$P\ \overline{E}\ (A)\ \overline{C}\ \overline{G}$	–	–	–	–
	$\overline{P}\ \overline{E}\ (A)\ C\ \overline{G}$	–	–	–	–
	$\overline{P}\ E\ (A)\ \overline{C}\ \overline{G}$	–	–	–	–
	$P\ \overline{E}\ (A)\ \overline{C}\ G$	–	–	–	–
	$\overline{P}\ E\ (A)\ \overline{C}\ G$	–	–	–	–
	$P\ E\ \overline{A}\ \overline{C}\ \overline{G}$	–	–	–	–
	$P\ E\ (A)\ \overline{C}\ \overline{G}$	8	15	–	–
	$P\ E\ \overline{A}\ \overline{C}\ G$	–	–	5	10
	$P\ E\ (A)\ \overline{C}\ G$	16	10	10	20
	$\overline{P}\ E\ (A)\ C\ G$	–	–	3	6
	$P\ E\ A\ \overline{C}\ G$	9	6	–	–
	$P\ E\ \overline{A}\ C\ G$	32	20	9	18
	$P\ E\ (A)\ C\ G$	23	14	8	16
	$P\ E\ A\ C\ G$	23	14	6	12

---

[a] Frequencies are given only for those risk patterns presented by more than 5% of the total number of subjects in a group.

[b] From Del Corno *et al*., 1980.

[c] ( ) = Uncertain risk.

$\overline{X}$ = Risk not present.

TABLE 5

Dermatologic Cases from July 10 to August 18[a]
(Crude incidence rates/10,000 inhabitants)

City	Zone A	Zone B	Zone R	Surrounding Areas
Meda	586 (Meda and Seveso)		120	20
Seveso		305	131	46
Cesano		78	50	14
Desio		9	8	
Other				5

[a] From Fara, 1976b.

pattern was observed in the territorial distribution of the chloracne cases, as shown in Table 6. Mapping the chloracne cases (Figure 6) indicates a wider geographic distribution of chloracne than expected from the known soil pollution pattern.

Additional data on possible human exposure were obtained through the study of animal mortality (Veterinary Report, 1980). At the time of the accident, 81,131 domestic animals lived in Zones A, B, and R: 24,885 rabbits; 55,545 poultry and other small animals; 349 cattle; 233 pigs; 49 horses; 21 sheep; and 49 goats. Poultry and rabbits were raised mainly for household consumption in Zones A, B, and R. Cattle (5 to 10 head per farm) were raised mainly for milk production. Mortality started, mostly among rabbits and poultry, some few days after the accident, and rose markedly within the first 2 weeks. By the end of August 1976, 3,281 animals were recorded as dead (Table 7). Rabbit mortality, by zone, is shown in Table 8. Rabbit deaths occurred at ∿ 75% of the farms in Zone A, at ∿ 22% of the farms in Zone B, and at ∿ 15% of the farms in Zone R. The number of dead rabbits was 31.9% of the total in Zone A, 8.8% in Zone B, and 6.8% in Zone R. On farms where rabbits died, mortality was 42% in Zone A, 23% in Zone B, and 16% in Zone R. Mortality was higher on farms where rabbits were fed grass gathered in contaminated areas at various distances from the plant. There is strong evidence that mortality was directly related to the distance from ICMESA where the grass was gathered. Farms where rabbits were fed commercial feed or fodder collected before the accident or far from ICMESA had a lower mortality rate.

TABLE 6

Chloracne Cases (No. and x 1,000 inhabitants) by Pollution Zones[a]

Pollution Zone	(1) Chloracne Sept.-Dec. 1976 "Early"		(2) Chloracne Feb.-Apr. 1977 "Late"		Chloracne (1) + (2)	
	No.	x1000	No.	x1000	No.	x1000
Zone A	46	63.01	15	20.55	61	83.56
Zone B	0	–	9	1.90	9	1.90
Seveso, Zone R	1	0.13	28	3.52	29	3.65
Meda, Zone R	0	–	20	4.98	20	4.98
Cesano, Zone R	0	–	13	0.87	13	0.87
Desio, Zone R	0	–	2	0.43	2	0.43
Seveso, Zone non A,B,R	0	–	13	1.68	13	1.68
Meda, Zone non A,B,R	0	–	14	0.90	14	0.90
Cesano M., Zone non A,B,R	0	–	8	0.50	8	0.50
Desio, Zone non A,B,R	0	–	5	0.18	5	0.18
Other cities	3		10		13	
Total	50		137		187	

---

[a] From Bisanti et al., 1980.

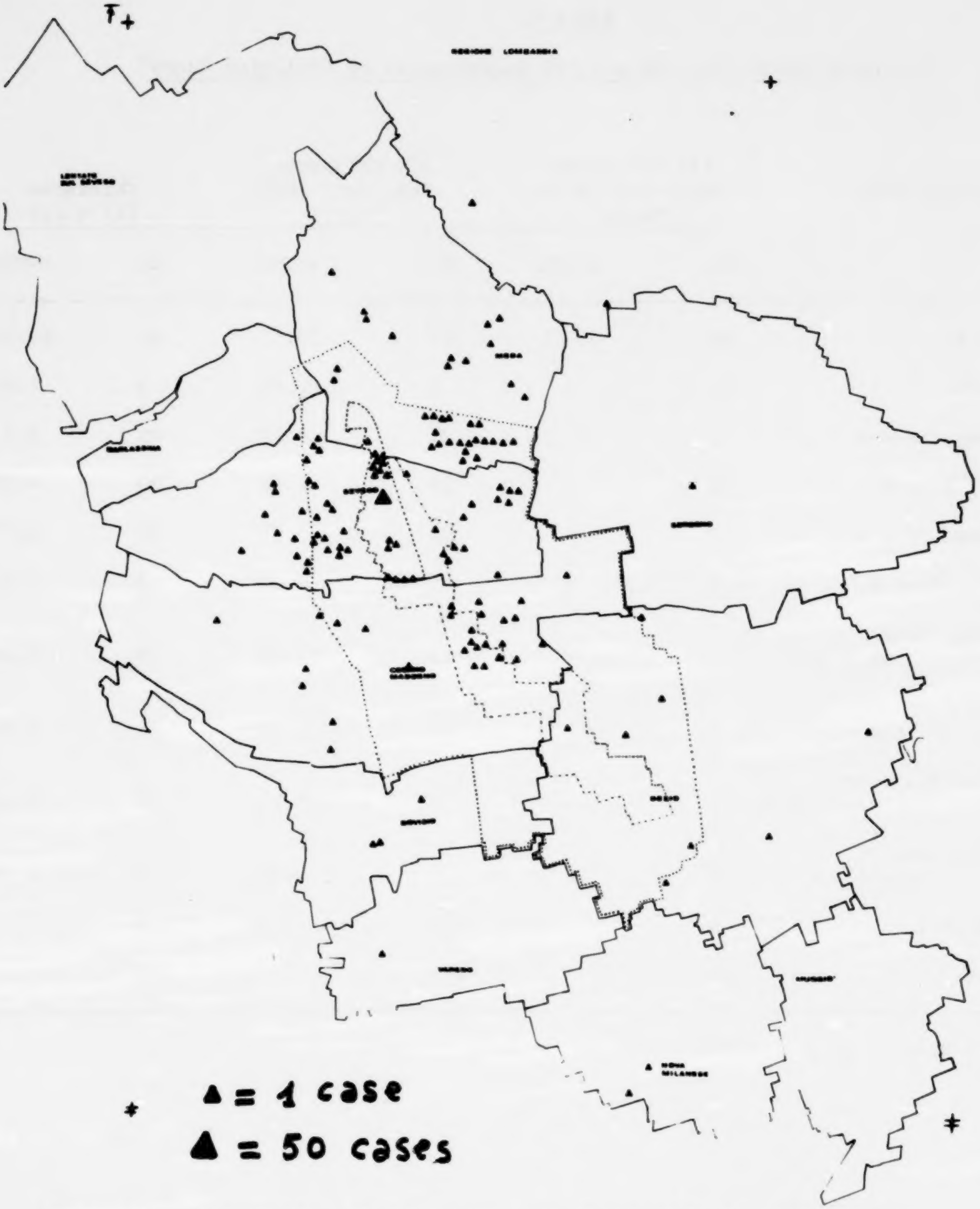

REGIONE LOMBARDIA
NOVA MILANESE
▲ = 1 case
▲ = 50 cases

FIGURE 6. Distribution of chloracne cases observed in Seveso area from July 10, 1976 to July 1977. From Del Corno _et al._, 1980.

TABLE 7

Overall Animal Mortality (July 10 - August 31, 1976)[a]

Animal	No. of dead animals/total
Rabbits	2,062/24,885
Other small farmyard animals	1,219/55,545
Cattle	0/349
Horses	0/49
Pigs	0/233
Sheep	0/21
Goats	0/49

[a] From Veterinary Report, 1980.

TABLE 8

Rabbit Mortality on Farms in the Contaminated Zones
July 10 - August 31, 1976[a]

Zone	No. of Rabbits			No. of Farms in Area			No. of Rabbits on Farms Where Deaths Occurred		
	Total	Dead	%	Total	Farms with Deaths	%	Total	Dead	%
A	1,089	348	31.9	45	34	75.5	825	348	42.2
B	4,814	426	8.8	303	67	22.1	1,801	426	23.6
R	18,982	1,288	6.8	1,398	208	14.9	7,783	1,288	16.5
Total	24,885	2,062	8.3	1,746	309	17.7	10,409	2,062	19.8

[a] From Veterinary Report, 1980.

During the first period, 926 autopsies were performed on 45% of the dead rabbits. Some 20% of these animals showed a syndrome characterized by substernal and retrosternal edema, hemmorrhagic tracheitis, pleural serous hemorrhage, and dystrophic lesions of hepatic tissue. The percentage of rabbits with pathologic syndrome was 44.4%, 21.4%, and 18.4% in Zones A, B, and R, as shown in Table 9. Some dead rabbits were analyzed for the presence of TCDD; 97%, 92%, and 75% of the samples were positive in Zones A, B, and R, respectively. Although fewer samples were analyzed for TCDD than were rabbits autopsied, it is clear that the presence of TCDD did not always correlate with the described pathologic syndrome.

Figure 7 shows the geographic distribution of animal deaths. These occurred on farms in all three zones and also in the surrounding areas. Of course, contaminated fodder may have been transported from Zone A to nearby areas, thereby reducing the significance of territorial distribution of animal deaths as an indicator of local TCDD pollution.

In summary, TCDD concentrations varied widely, even from site to site, within the regions studied. Animal mortality was correlated with a farm's distance from the ICMESA plant, or with the distance of the source of the animal's food (grass or fodder) from the plant. Incidence of chloracne in children was not related to exposure indicators. The disease occurred over a wider geographic area than was expected from the known TCDD soil contamination. Acute cutaneous lesions were more frequent in residents of Zone A than in residents of Zones B and R, but residents of Zone R did not exhibit fewer such lesions than did those of Zone B, as was expected.

TABLE 9

Autopsies on Rabbits (July - August, 1976)[a]

Zones	No. of Dead Rabbits	No. of Autopsies	%	No. of Rabbits with Pathological Syndrome[b]	% of Examined Rabbits
A	348	89	26	36	40.4
B	426	196	46	42	21.4
R	1,288	641	50	118	18.4
Total	2,062	926	45	199	21.5

[a] From Veterinary Report, 1980.

[b] Substernal and retrosternal edema, hemorrhagic tracheitis, pleural serous hemorrhage, dystrophic lesions of hepatic tissue.

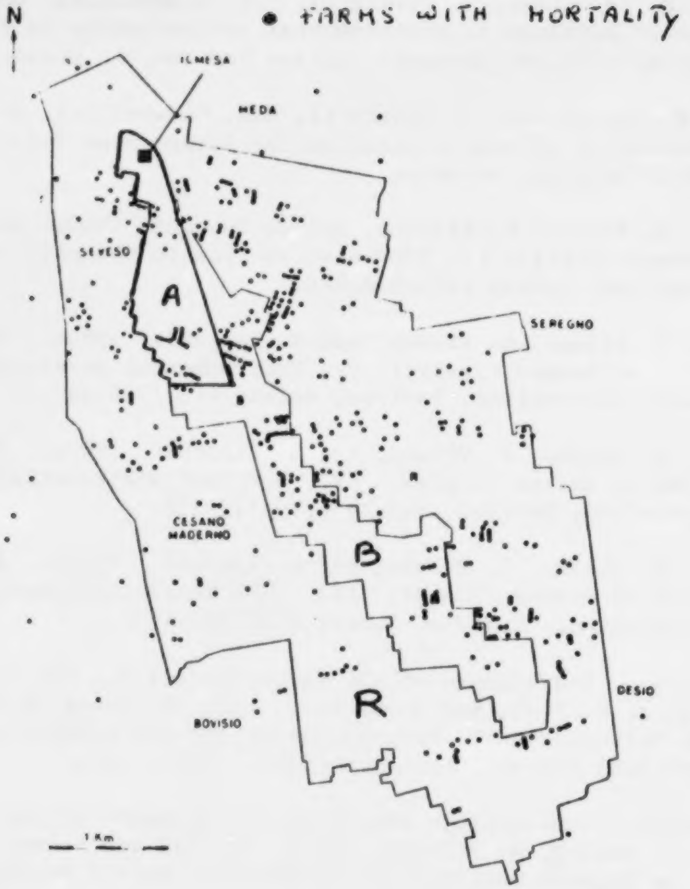

N
● FARMS WITH MORTALITY
ICMESA
MEDA
SEVESO
A
SEREGNO
CESANO MADERNO
B
R
DESIO
BOVISIO
1 Km

FIGURE 7. Mapping of animal deaths. From Veterinary Report, 1980.

REFERENCES

Bisanti, L., F. Bonetti, F. Caramaschi, G. Del Corno, C. Favaretti, S.E. Giambelluca, E. Marni, E. Montesarchio, V. Puccinelli, G. Remotti, C. Volpato, E. Zambrelli, G.M. Fara. 1980. Experiences from the accident of Seveso. Acta Morphol. Acad. Sci. Hung. 28(1-2):139-157.

Caramaschi, F., G. Del Corno, C. Favaretti, S.E. Giambelluca, and E. Montesarchio. 1981. Analysis of exposure to environmental contamination by TCDD in individuals affected by dermatological lesions. Igiene Moderna, in press.

Del Corno, G., F. Caramaschi, C. Favaretti, S.E. Giambelluca, and E. Montesarchio. 1981. Distribution of chloracne cases in the Seveso area following contamination by TCDD. Igiene Moderna, in press.

DiDomenico, A., V. Silano, G. Viviano, and G. Zapponi. 1980a. Accidental release of TCDD at Seveso (Italy): II. TCDD distribution in the soil surface layer. Ecotoxicol. Environ. Safety 4(3):238-320.

DiDomenico, A., V. Silano, G. Vivano, and G. Zapponi. 1980b. Accidental release of TCDD at Seveso (Italy): V. Environmental persistence (half-life) of TCDD in soil. Ecotoxicol. Environ. Safety 4(3):339-345.

DiDomenico, A., V. Silano, G. Vivano, and G. Zapponi. 1980c. Accidental release of TCDD at Seveso (Italy): IV. Vertical distribution of TCDD in soil. Ecotoxicol. Environ. Safety 4(3):327-338.

DiDomenico, A., V. Silano, G. Vivano, and G. Zapponi. 1980d. Accidental release of TCDD at Seveso (Italy): VI. TCDD levels in atmospheric particles. Ecotoxicol. Environ. Safety 4(3):346-356.

Fara, G. M. 1976a. The history of the Seveso accident. Pp. 171-79 in E. B. van Julsingha, J. M. Tesh, and G. M. Fara, eds. Advances in the Detection of Congenital Malformations: Proceedings of the 5th Conference of the European Teratology Society, Gargnano, Sept. 20-23, 1976.

Fara, G. M. 1976b. Introductory report on the epidemiological aspects. Pp. 39-46 in A. Berlin, A. Buratta, and M. Th.-Van der Venne, eds. Proceedings of the Experts Meetings on the Problems Raised by TCDD Pollution, Milano, Sept. 30-Oct. 1976. Regione Lombardia.

Pocchiari, F., V. Silano, and A. Zampieri. 1979. Human health effects from accidental release of TCDD at Seveso, Italy. Pp. 311-21 in W. J. Nicholson and J. A. Moore, eds. Volume 320, Human Effects of Halogenated Aromatic Hydrocarbons. New York Academy of Science, New York.

Regione Lombardia, Ufficio Speciale. 1980. Veterinary Report, offset print.

DISCUSSION

DR. MILLER: Dr. Pocchiari has summarized four major problems related to chemical accidents at the administrative and scientific levels: uneven soil distribution, difficulties in monitoring, lack of rapid assay methods, and problems in coordinating the jurisdictions. Your study, specific to Seveso, has shown that TCDD has a long half-life (more than 10 years), that you could make some clinical observations on the basis of experience with TCDD (there are other chemicals for which there is no past experience), that "heavily exposed" must be defined specifically, and that you had an advantage in that you could observe effects in domestic animals -- rabbits -- acting as biologic indicators. Autopsies of the rabbits provided you with chemical analyses.

DR. SUSKIND: Did the group in Seveso regard chloracne as a clinical marker? Did the appearance of acne signal exposure to a degree that adverse effects occurred, or were there other adverse effects and other incidents? How severe were the acne cases? In the United States, we have heard all kinds of stories about the mildness of acne and the late occurrence of acne in children.

PROF. DARDANONI: Chloracne is presently the only indicator we have of human morbidity. Therefore, we rely on it to make a correlation between soil distribution and human pathology. Severity was rather strong in the first group of people. Late chloracne cases were much less severe. There was a spectrum partly correlated with distance of the child's residence from the ICMESA plant.

DR. MOORE: You suggested that the initial half-life of TCDD was about 3 or 4 months, and now you are talking about a half-life extending into years. Is time a function of the chemical's location in the soil?

PROF. SILANO: These data applied only to Zone A and only to the worked soil from this zone. Zone A was evacuated just 2 weeks after the accident. Since then, it has not been worked, and no people are in the zone.

This study was not a special activity to evaluate TCDD's half-life in soil. It was an attempt to record different monitoring at different times and to get some idea of the persistence of TCDD in soil.

We had three monitorings; the first about 1 month after the accident, the second after approximately 5 months, and the last one after 14 or 15 months. We were able to compare TCDD levels at 44 sites, of which 32 were identical.

We made two estimates. The first was based on 44 sites, and the second on 32. At the initial monitoring, there were no siginificant or relevant differences among these estimates. TCDD levels decreased about 50% on the average (basically a geometric mean) between the first and the second monitorings, but we found no statistically significant difference between the second and third monitorings.

The mathematic function that describes the overall pattern of results indicates that the TCDD half-life in soil changes with time. In other words, half-life is not a constant value. In fact, from the mathematic function, we calculated that, in measurements made at the times I described, the half-life in this unworked soil is more than 10 years.

The literature has reported substantial differences in the half-life of TCDD in different experiments, ranging from a few months up to 3 or more years. The data at Seveso may explain the variability. Certainly they suggest several hypotheses.

TCDD in the very top soil or even on plants and leaves and so on may be degraded by sunlight. After the chemical moves down into the soil, it is not degraded by sunlight, and this fact is certainly important.

Italy received exceptionally heavy rainfalls between August and December; so some TCDD certainly penetrated the soil. TCDD really did move downward. This may be not only because TCDD on the very surface was destroyed, but also because at the moment we can say that the upper layer of 20 cm contains most of the TCDD. Soon after the contamination occurred, the soil layer containing 80% TCDD was much less; it was only 8 cm deep. So, there has since been some deeper penetration into the soil.

A second hypothesis concerns volatilization of TCDD. The soil is a very heterogeneous matrix, and one would expect TCDD, with time, to be redistributed among the soil's colloid matrix and different components. But stronger bonds seem to develop and prevent volatilization. Both sunlight and increased resistance to disease may explain our results.

Yusho (Japan)

Robert W. Miller[1]

Yusho (rice-oil) disease was first recognized because of an epidemic of chloracne that began in February 1968 in Kyushu, Japan. About 1,200 people were affected; their illnesses were quickly traced to polychlorinated biphenyls (PCB's) used as a heat transfer agent during the manufacture of cooking oil. Nine women, pregnant at the time of their exposure, subsequently gave birth to infants who were small-for-date and had transiently cola-colored skin. Followup of the affected population, thus far, has not revealed carcinogenesis or other adverse health effects related to the PCB's. A similar leak occurred soon afterwards in the United States; chickenfeed was polluted with PCB's, but no human exposure was reported. Damage was revealed in reduced hatchability of the eggs. A second chloracne epidemic developed recently in Taiwan, again traced to cooking oil contaminated with PCB's during manufacture. The lactational transmission of the substance observed in this instance has raised questions about the advisability of breastfeeding by women occupationally or otherwise exposed to PCB's.

Approximately 1,200 people around the city of Fukuoka, on the Japanese island of Kyushu, developed chloracne from exposure to an environmental pollutant (Taki *et al*., 1969). The cause was quickly traced to cooking oil, which was contaminated with polychlorinated biphenyls (PCB's) used as a heat transfer agent during manufacture of the oil. In addition to chloracne developed by the general population, infants born to women who ingested the oil during pregnancy were also affected, indicating placental transfer of the agent. Doses of PCB's received by the women were estimated through chemical analyses of the cooking oil and from the history of of its ingestion by the family. The illness was termed Yusho (rice oil) poisoning.

---

[1] Clinical Epidemiology Branch, National Cancer Institute, Bethesda, Md.

Nine cola-colored infants (as the Japanese described them) were born with skin transiently stained a deep brown. The discoloration cleared with time. All of the infants were exposed *in utero*, and were small-for-date. In addition, two were born with teeth, a finding that warrants additional thought.

According to Harada (1976), Children developed chloracne after ingesting PCB's in breast milk from women who were exposed to the chemical only after their pregnancies had ended. Breast milk samples were not studied during the Kyushu epidemic. Generally speaking, the levels of PCB's in breast milk in Japan are no higher than they are in the United States (Tatsukawa, 1976).

The experience in Japan heralded PCB problems elsewhere in the world. In the United States, Holly Farms, Inc., which is a supplier of chickens and eggs, discovered reduced hatchability of its eggs, and traced the problem to PCB contamination of chickenfeed (Pichirallo, 1971). The contamination occurred in a manner similar to that of the Japanese cooking oil. PCB's, used as a heat-transfer agent, leaked into the feed through pin-hole erosions in the pipes.

Also in the United States, the Hudson River and some of the Great Lakes were polluted with PCB's leaking from capacitors discarded by General Electric Co. into the waterways (Dennis, 1976). At the same time, those areas were being stocked with salmon for sport-fishing.

Another epidemic (1,000 cases) of chloracne has recently been reported by Chen *et al*. (1980). Again, the source was contaminated cooking oil.

Thus, PCB's have caused areawide food contamination, which may have a transplacental effect and produce chloracne. They may also be transmitted in breast milk. To date, effects in the general population have been limited to the skin and the fetus.

REFERENCES

Chen, P.H., J.M. Gaw, C.K. Wong, and C.J. Chem. 1980. Levels and gas chromatographic patterns of polychlorinated biphenyls in the blood of patients after PCB poisoning in Taiwan. Bull. Environ. Contam. 25:325-329.

Dennis, D.S. 1976. Polychlorinated biphenyls in the surface waters and bottom sediments of the major drainage basins of the United States. Pp. 183-194 in National Conference on Polychlorinated Biphenyls (November 1975, Chicago, Illinois), EPA-560/6-75-004, Environmental Protection Agency, Washington, D.C.

Harada, M. 1976. Intrauterine poisoning: Clinical and epidemiological studies and significance of the problem. Bull. Inst. Constitutional Med. (Kumamoto Univ.) Suppl. 25: 1-60.

Pichirallo, J. 1971. PCBs: Leaks of toxic substances raises issue of effects, regulations. Science 173:899-902.

Taki, I., S. Hisanaga, and Y. Amagase. 1969. Report on Yusho (chlorobiphenyls poisonings): Pregnant women and their fetuses. Fukuoka Igaku Zasshi 60:471-474.

Tatsukawa, R. 1976. PCB pollution of the Japanese environment. Pp. 173, 177-78 in K. Higuchi, ed. PCB Poisoning and Pollution. Academic Press, New York.

DISCUSSION

DR. SUSKIND: As a dermatologist, I have seen some of the chloracne cases at Kyushu University, where there has been an attempt to follow the mothers and children. There are some reports that second pregnancies 1 year or more after the mother's exposure to PCB's seem to result in darker than usual--hypermelanotic-infants. In addition, the chloracne of patients first seen in 1968 persisted for 2 or 3 years.

DR. MILLER: On the Goto Islands, two or three children have been affected in some families in which several children had been born subsequent to the PCB exposure. The effect has, however, diminished in successive pregnancies. The Kyushu mothers who had an affected child have not had additional children. (Because of Catholicism on the Goto Islands, birth control is not practiced there as it is on Kyushu.)

One more comment: Some international organization may want to watch for similar occurrences with PCB's or other substances, so that they can be brought promptly to international attention and their implications can be recognized. The transmission of the first information on PCB's was by coincidence.

DR. NEWELL: The exposure in Kyushu is very interesting, in that it was prolonged--as opposed to exposures to other agents, which are usually single, relatively short-term events. Is there any information on the concentrations of PCB's or on the duration of exposure?

DR. MILLER: I do not know, although a report by Kuratsune (1976) states that two mothers were exposed throughout their pregnancies and that the others were exposed for only a few weeks.

REFERENCE FOR DISCUSSION

Kuratsune, M. 1976. Epidemiologic studies on Yusho. P. 20 in K. Higushi, ed. PCB Poisoning and Pollution. Academic Press. New York.

Treatment of Chlordecone (Kepone) Poisoning with Cholestyramine

Philip S. Guzelian [1]

Due to the lack of adequate industrial hygienic protection, workers in a factory that manufactured chlordecone as its only product were exposed to large quantities of the organochlorine pesticide for many months. The salient clinical features in 32 of the most severely affected workers, all males, involved the nervous system, liver, and testes. High levels of chlordecone were found in samples of blood, adipose tissue, and liver. However, the pesticide was absent from excretory fluids (sweat, urine, and sebum), but present in gallbladder bile. Only 5-10% of the biliary chlordecone appeared in the stool, which suggested that chlordecone may be reabsorbed in the intestine. Oral administration of cholestyramine, a nonabsorbable anion exchange resin that binds chlordecone *in vitro*, increased the fecal elimination of chlordecone from the body and accelerated its disappearance. Recent evidence indicates that chlordecone enters the intestine by an alternate, nonbiliary mechanism (probably via the gut itself), and that net excretion of chlordecone from this source can be augmented by cholestyramine administration. Coincident with elimination of chlordecone from the tissues was an amelioration of the signs and symptoms of chlordecone toxicity. Since other lipophilic toxins also appear to be excreted by this mechanism, oral administration of selected binding agents may provide a practical, therapeutic approach to treating poisonings from many types of environmental chemicals.

In July 1975, a chemical worker for Life Science Products Corporation, Hopewell, Va., which manufactured chlordecone (Kepone) as its only product, was discovered to have high concentrations of that chemical in his blood and severe toxic manifestations involving his neurologic system, liver, and testes (Cohn *et al.*, 1978). Epidemiologic followup of this index case by Federal and State health officials revealed that the small factory consisted of a renovated gasoline service station, which lacked adequate industrial hygienic protective measures and employee safety provisions (Cannon et al., 1978). These conditions led to frequent exposure of the workers to large quantities of the organochlorine pesticide. Indeed, two-thirds of the production workers employed for all or a portion of the 16 months the factory was in operation exhibited clinical evidence of chlordecone toxicity, and almost all had detectable concentrations of chlordecone in blood samples (Table 1). The production workers wore no special protective clothing and took their work clothes home, which probably explains the contamination of their family members who also had detectable chlordecone in blood samples (Table 1). Because the factory discharged chlordecone into the air and water, the city's sewage system became contaminated and sewage workers and residents of the area were also exposed to chlordecone (Table 1).

---

[1] Medical College of Virginia, Richmond, Va.

It was learned subsequently that Allied Chemical Corporation, which had been the exclusive manufacturer of chlordecone prior to its contractual arrangement with Life Science Products Corporation, had discharged the pesticide into the James River for more than 10 years. Evidence of contamination of the animal and marine life of the entire tidewater region of Virginia has been gathered, but the total number of individuals at risk of low-dose exposure to chlordecone in the environment is presently unknown.

A team of clinical investigators studied a group of 32 of the most severely affected men, aged 18 to 47. Each had signs or symptoms (or both) consistent with chlordecone toxicity and a concentration

TABLE 1

Epidemiology of Chlordecone Poisoning at Life Science Products Corporation, Hopewell, Va.[a]

Finding	Subjects (No. Affected/No. Studied)	Percent Affected
Attack Rate:	Production workers (73/114)	67
Chlordecone detected in blood:	All workers (105/106)	99
	Family members (30/32)	94
	Residents of Hopewell (40/214)	19

---

[a] Data from Cannon *et al.*, 1978.

of chlordecone in the blood greater than 600 ng/ml. Evidence of neurologic impairment included a motor disorder manifested by appendicular tremor, stuttering speech, and opsoclonia (arrhythmic eye movements). Also frequent were findings of anxiety, short-term memory loss, change in personality, and headaches. In the majority of these patients, the liver was enlarged, although chemical tests of liver function showed normal results. Morphologic examination of liver biopsies revealed proliferation of the smooth endoplasmic reticulum as the most consistent finding. Evidence for impairment of testicular function included oligospermia and a decreased percentage of motile sperm. The salient features of the clinical syndrome exhibited by these men have been described by Cohn et al. (1978) and Taylor et al. (1978).

No cases of human poisoning with chlordecone had been reported previously, and no specific treatment for this condition was available. Indeed, although toxicity and even death have occurred in humans exposed to chlorophenothane (DDT) (Case, 1945; Hill and Robinson, 1945; Jenkins and Toole, 1964; Mackerras and West, 1946; Wigglesworth, 1945), dieldrin (Garrettson and Curley, 1969; Hoogendam et al., 1965; Jindal, 1968; Patel and Rao, 1957), aldrin (Hoogendam et al., 1965; Jenkins and Toole, 1964; Kazantizis et al., 1964; Spiotta, 1951), endrin (Coble et al., 1967; Davies and Lewis, 1956), chlordane (Curley and Garrettson, 1969; Derbes et al., 1955), polychlorinated biphenyls (Fishbein, 1974; Hirayama, 1976), and polybrominated biphenyls (Kay, 1977), no antidotes are capable of reversing the toxic lesions created by these organochlorine chemicals.

One approach to therapy was to devise a means to accelerate chlordecone excretion from the body, on the assumption that the substance's presence in the tissues is responsible for toxic manifestations, constitutes a risk of carcinogenesis, or both. The issue of carcinogenesis was raised by studies demonstrating that chlordecone is a hepatic carcinogen in rats and mice (Reuber, 1978). Accordingly, a study of the distribution and excretion of chlordecone in these patients revealed that the pesticide is excreted into the intestine by the liver bile (Cohn _et_ _al_., 1978) and also by a novel, nonbiliary mechanism (Boylan _et_ _al_., 1979). Based on this information and current concepts of gastrointestinal physiology, Guzelian _et_ _al_. (1980) established that oral administration of cholestyramine, a nonabsorbable anion exchange resin that binds chlordecone _in_ _vitro_, increases the excretion of the pesticide in the stool and accelerates its elimination from tissues, thereby ameliorating the clinical manifestations of toxicity.

METHODS

Details of the patient population, and the methods used to obtain and prepare samples of blood, fat, bile, and stool have been published elsewhere (Blanke _et_ _al_., 1977; Boylan _et_ _al_., 1979; Cohn _et_ _al_., 1978; Guzelian _et_ _al_., 1980). Chlordecone was measured by gas-liquid chromatography using an electron-capture detector.

RESULTS

Distribution and Excretion of Chlordecone and Effects of Cholestyramine Treatment

The highest concentrations of chlordecone were found in the blood, liver, and adipose tissue (Table 2). The ratio of the concentration of chlordecone in the blood compared to its concentration in fat (approximately 1:7) is extremely high when compared to the ratio reported for other organochlorine pesticides. One explanation for this phenomenon is the specific binding of chlordecone by plasma proteins, particularly by high-density lipoproteins (Skalsky *et al.*, 1979). A practical consequence of this unusual distribution is that the measurement of chlordecone in blood is a far more sensitive indicator of exposure than would be expected for other organochlorine pesticides. Indeed, across a 5,000-fold range of chlordecone concentrations in adipose tissue, a strict ratio of 7:1 was maintained with the respective blood concentration (Cohn *et al.*, 1978).

Of excretory fluids examined (sweat, urine, saliva, gastric juice), only gallbladder bile contained significant quantities of chlordecone (Table 2). The rates of excretion of chlordeconed in hepatic bile, measured by aspiration of duodenal contents, represented a relatively constant proportion (0.5-1.0%) of the estimated total body content of chlordecone (Cohn *et al.*, 1978). An important finding, however, was that only 5-10% of the biliary chlordecone entering the lumen of the duodenum appeared in the feces each day. This discovery suggested that 90-95% of the biliary chlordecone was reabsorbed in the intestine and recirculated to the liver, thus undergoing enterohepatic recirculation.

TABLE 2

Distribution of Chlordecone in Workers[a]

Tissue	No. of Patients	Range of Chlordecone Concentration (μg/g)	Partition Blood Tissue	Range
Whole blood	32	0.6-32.0	1.0	--
Liver	10	13.3-173.0	15.0	4.6-31.0
Subcutaneous fat	29	1.7-62.1	6.7	3.8-12.2
Muscle	5	1.2-11.3	2.9	1.8-4.5
Gallbladder bile	6	2.5-30.0	2.5	1.4-4.1

---

[a] From Cohn *et al.*, 1978; reprinted by permission of *The New England Journal of Medicine* 298:243-248, 1978.

To test the hypothesis that cholestyramine, which binds chlordecone _in vitro_, might also bind this substance in the intestine, the investigators administered this resin orally (24 g/day) to nine patients for 5 days. There were marked increases in the fecal excretion of chlordecone, ranging from 3.3-fold to 17.8-fold (Figure 1). By comparison, oral administration of activated charcoal to three patients produced only a minimal increase in fecal excretion of chlordecone. Similarly, a 2-week cholestyramine-feeding study in chlordecone-treated rats established that the resin not only increased fecal excretion of the pesticide, but also decreased total body content of chlordecone (Boylan _et al_., 1978).

Encouraged by these preliminary studies, the researchers carried out a double-blind, randomized clinical trial of cholestyramine therapy (Cohn _et al_., 1978). Patients were classed into three groups, according to the concentration of chlordecone in their blood when they entered the study. Within each group, the men were randomly allocated to treatment with either cholestyramine or placebo, and were observed twice monthly for 5 months. Cholestyramine significantly accelerated the rate of disappearance of chlordecone from the blood (Figure 2) and also from adipose tissue (Cohn _et al_., 1978).

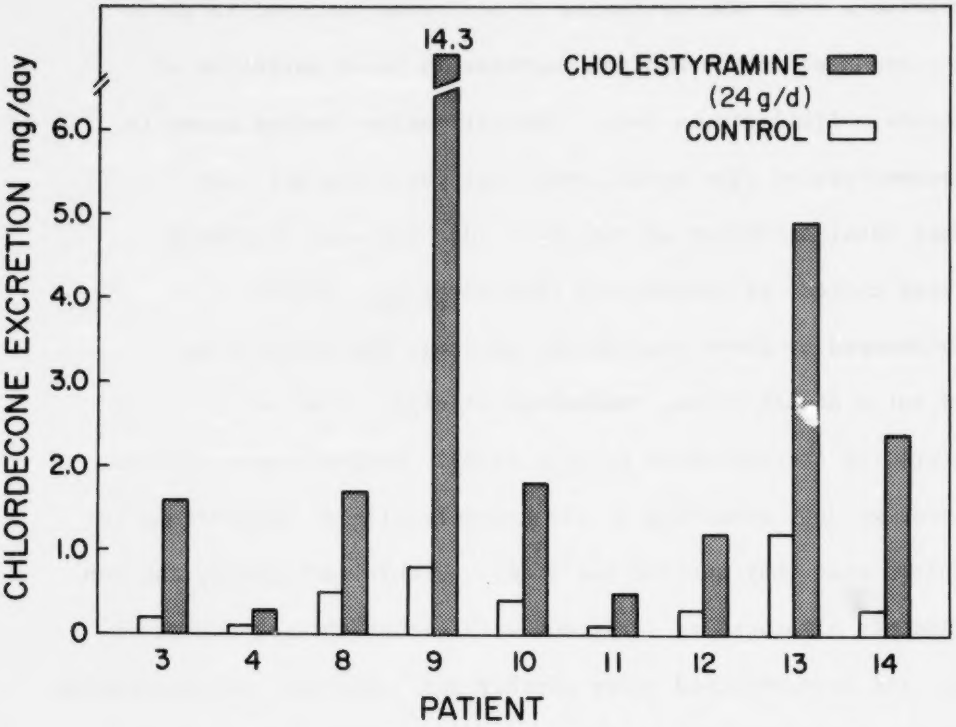


FIGURE 1. Stimulatory effect of cholestyramine on the rate of excretion of chlordecone in stool. Control collections of stool were obtained from each patient for at least 72 hours. Treatment with cholestyramine was started and, 48 hours later, stool was collected for a second 72-hour period. From Cohn et al., 1978, with permission from The New England Journal of Medicine 298:243-248, 1978.

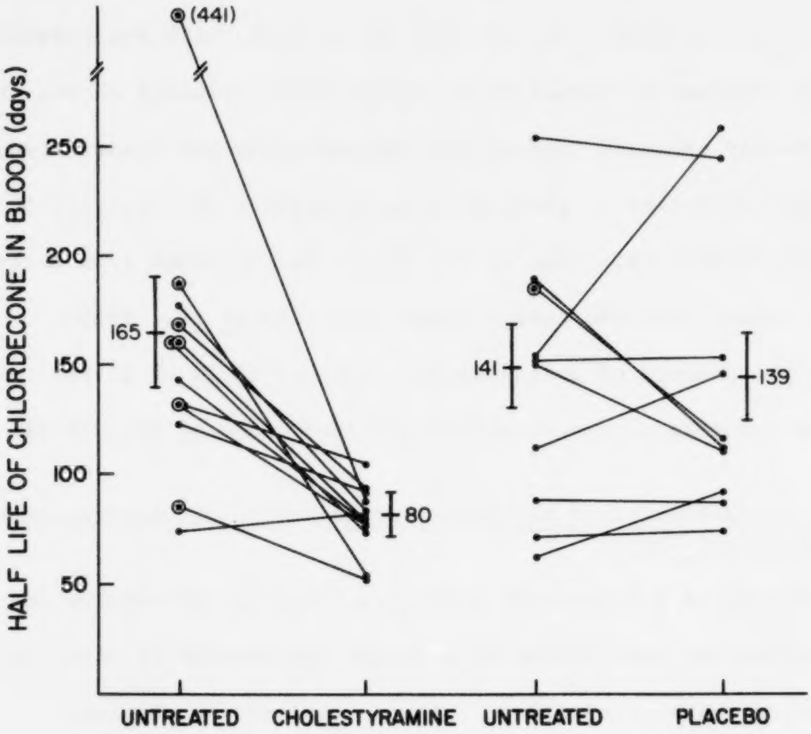


FIGURE 2. Effect of cholestyramine or placebo on the half-life of chlordecone in the blood of exposed workers. Open circles denote patients with statistically significant differences between the half-lives of chlordecone in the control period (untreated) and the treatment period ($p < 0.05$). Average half-lives are given as mean ± S.E.M. (brackets). From Cohn *et al.*, 1978, reprinted with permission from *The New England Journal of Medicine* 298:243-248, 1978.

Amelioration of Toxic Manifestations with Cholestyramine Treatment

After the clinical trial, all patients were placed on cholestyramine. Within 1 year, their blood showed low or undetectable concentrations of chlordecone. Coinciding with a decrease in chlordecone levels in blood (and presumably in total body content) was the disappearance of objective neurologic findings. All but one of the patients was able to return to work. In addition, in patients with hepatomegaly, the liver returned to normal size, repeat liver biopsies showed that the previously observed nonspecific abnormalities had disappeared, and urinary excretion of glucaric acid (a measure of induction of smooth endoplasmic reticulum in the liver) had declined from markedly elevated values into the normal range (Guzelian *et al.*, 1980). Finally, the numbers of motile sperm increased in 12 of 13 patients, and blood chlordecone concentration declined (Cohn *et al.*, 1978).

Evidence for a Nonbiliary Mechanism for Excretion of Chlordecone by the Gastrointestinal Tract

Discovery of a nonbiliary mechanism for entry of chlordecone into the intestine was facilitated by a unique opportunity to study biliary excretion of chlordecone directly in one patient who required cholecystectomy for gallstones and agreed to have a T-tube implanted in his common bile duct. Details of the experiments have been published by Boylan *et al.* (1979). Pertinent data are summarized in Table 3. Hepatic bile was diverted through the T-tube and small samples were taken for analysis of chlordecone and its metabolite, chlordecone alcohol. The rest of the bile was reinfused into the duodenum through

TABLE 3

Excretion of Chlordecone in T-Tube Bile and Stool in a Single Patient[a]

Condition	Bile Excretion (μg/24 hr)		Stool Excretion (μg/24 hr)	
	Chlordecone	Chlordecone alcohol	Chlordecone	Chlordecone alcohol
Intact circuit (bile reinfused)	593	486	88	195
Interrupted circuit (bile diverted)	258	250	240	<5

---

[a] For each condition, bile was collected for 24 hours during the second day of a 72-hour stool collection; bile was either saved or infused into the duodenum through a surgically implanted tube (Boylan *et al.*, 1979). Chlordecone and Chlordecone alcohol were analyzed by modifying a previously described method (Boylan *et al.*, 1979; Blanke *et al.*, 1977) in that samples were digested with β-glucuronidase and then autoclaved (115°C at 20 psi for 15 min) in the presence of sulfuric acid (10% final concentration). This treatment appeared to release Chlordecone, producing higher values than those obtained using the previous method (Boylan *et al.*, 1979). Data are the average of two experiments.

a surgically implanted tube. Under these conditions (enterohepatic recirculation intact), only 15% of the biliary chlordecone appeared in the stool each day, confirming earlier studies using duodenal aspiration techniques (Cohn et al., 1978). Similarly, chlordecone alcohol seemed to undergo enterohepatic recirculation, although to a lesser extent (60% reabsorbed). When bile diverted from the T-tube was not reinfused (thus, interrupting enterohepatic recirculation), chlordecone alcohol failed to appear in the stool (Table 3). This finding is consistent with the interpretation that chlordecone alcohol is formed in the liver and that bile is the sole source of the chlordecone alcohol in the feces. In contrast, such diversion of the bile stream did not reduce or terminate fecal excretion of chlordecone. Indeed, the excretion of chlordecone in stool increased following biliary diversion (Table 3). This observation, confirmed in rats (Boylan et al., 1979), establishes the existence of a nonbiliary mechanism (probably the gut itself) for entry of chlordecone into the intestine. Moreover, it may be inferred that constituents in bile inhibit the net transportation of chlordecone into the lumen by this nonbiliary pathway (Table 3).

OVERVIEW

Polyhalogenated hydrocarbon chemicals have been produced and used in steadily increasing amounts during the last 25 years. As a consequence, they have become ubiquitous contaminants of our environment. There is little specific information on the pathobiologic effects of environmental chemicals in humans, perhaps because potential adverse health effects of chronic exposure to low doses of these substances are difficult to study. The unfortunate industrial exposure of chemical workers to chlordecone provides several unique advantages for investigating these effects. The workers were all young, adult males and were exposed to a single chemical, at high doses, over a well-defined period of time.

Blanke _et_ _al_. (1977) first developed and validated a method to measure chlordecone in biologic samples. Methods for analysis of environmental chemicals often are validated by samples "spiked" with standards. This technique may be inappropriate for measurements of chlordecone and probably for many other lipophilic chemicals in biologic samples. Extraction of chlordecone from excreta or tissues of animals treated with ^{14}C-chlordecone differs significantly from samples of the same tissues spiked with exogenously added standards. Indeed, amounts of chlordecone extracted from biologic samples varies widely among different tissues and may even differ among replicate samples within a given tissue. Whereas appropriate external standards (monohydrochlordecone) can correct for the latter variation, careful analysis of endogenously labeled samples is necessary to correct for the former.

The researchers determined that chlordecone was stored in the highest concentrations in adipose tissue and in the liver and was excreted in bile into the gastrointestinal tract. However, there is a substantial enterohepatic recirculation of chlordecone, which curtails the overall elimination of the pesticide from the body. Accordingly, the hypothesis that cholestyramine might block the reabsorption of chlordecone in the intestine and increase its excretion in the stool was tested. Both short-term administration of cholestyramine to humans and animals, as well as a long-term controlled clinical trial of cholestyramine verified this hypothesis. Coincident with chemical detoxification of the patients was an amelioration of the signs and symptoms of chlordecone toxicity. This finding implies that the clinical manifestations of toxicity sustained in some patients for the 16 months they worked at Life Science Products Corporation and for many months thereafter were not irreversible.

The discovery of the nonbiliary mechanism for entry of chlordecone into the intestine was made possible by an opportunity to measure directly the excretion of an organochlorine chemical in human bile. One important feature of the nonbiliary mechanism is that more chlordecone undergoes net translocation into the intestinal lumen via this pathway than by biliary excretion. Thus, the intestine should be considered as a primary excretory organ as well as a conduit for products of hepatic metabolism. A second important feature of the nonbiliary pathway is that it may transport chemicals other than chlordecone. In monkeys and rats, halogenated chemicals such as mirex (Pittman *et al*., 1976), dieldrin (Heath and Vadecar, 1964), and polychlorinated biphenyls (Yoshimura and Yoshihara, 1975), as well as endogenous lipophilic substances such as unconjugated bilirubin (Lester *et al*., 1962), are excreted in the stool in larger quantities than in bile. Thus, a variety of hydrophobic chemicals, including those that are poorly translocated into bile can be eliminated via the nonbiliary route. If the precise mechanisms of nonbiliary transport can be elucidated and suitable binding agents developed for these chemicals, then promising means may be at hand to enhance the removal of slowly excreted lipophilic toxins from the body.

REFERENCES

Blanke, R.V., M.W. Fariss, F.D. Griffith, Jr., and P.S. Guzelian. 1977. Analysis of chlordecone (Kepone) in biological specimens. J. Analyt. Toxicol. 1:57-62.

Boylan, J.J., W.J. Cohn, J.L. Egle, Jr., R.V. Blanke, and P.S. Guzelian. 1979. Excretion of chlordecone by the gastrointestinal tract: Evidence for a nonbiliary mechanism. Clin. Pharmacol. Ther. 25:579-585.

Boylan, J.J., J.L. Egle, Jr., and P.S. Guzelian. 1978. Use of cholestyramine as a new therapeutic approach for chlordecone (Kepone) poisoning. Science 199:893-895.

Cannon, S.B., J.M. Veazey, Jr., R.S. Jackson, V.W. Burse, C. Hayes, W.E. Straub, P.J. Landrigan, and J.A. Liddle. 1978. Epidemic Kepone poisoning in chemical workers. Am. J. Epidemiol. 107:529-537.

Case, R.A.M. 1945. Toxic effects of 2,2-bis(p-chloro-phenyl)1,1,1-trichlorethane (DDT) in man. Br. Med. J. 2:842-845.

Coble, Y., P. Hildebrandt, J. Davis, F. Raasch, and A. Curley. 1967. Acute endrin poisoning. J. Am. Med. Assoc. 202:489-493.

Cohn, W.J., J.J. Boylan, R.V. Blanke, M.W. Fariss, J.R. Howell, and P.S. Guzelian. 1978. Treatment of chlordecone (Kepone) toxicity with cholestyramine: Results of a controlled clinical trial. N. Engl. J. Med. 298:243-248.

Curley, A., and L.K. Garrettson. 1969. Acute chlordane poisoning. Arch. Environ. Health 18:211-215.

Davies, G.M., and I. Lewis. 1956. Outbreak of food poisoning from bread made of chemically contaminated flour. Br. Med. J. 2:393-398.

Derbes, V.J., J.H. Dent, W.W. Forrest, and M.F. Johnson. 1955. Fatal chlordane poisoning. J. Am. Med. Assoc. 158:1367-1369.

Fishbein, L. 1974. Toxicity of chlorinated biphenyls. Ann. Rev. Pharmacol. 14:139-156.

Garrettson, L.K., and A. Curley. 1969. Dieldrin: Studies in a poisoned child. Arch. Environ. Health 19:814-822.

Guzelian, P.S., G. Vranian, J.J. Boylan, W.J. Cohn, and R.V. Blanke. 1980. Liver structure and function in patients poisoned with chlordane (Kepone). Gastroenterology 78:206-213.

Heath, D., and M. Vadecar. 1964. Toxicity and metabolism of dieldrin in rats. Br. J. Ind. Med. 21:269-279.

Hill, K.R., and G. Robinson. 1945. A fatal case of DDT poisoning in a child. Br. Med. J. 2:845.

Hirayama, C. 1976. Clinical aspects of PCB poisoning. Pp. 87-102 in K. Higuchi, ed. PCB Poisoning and Pollution. Academic Press, New York.

Hoogendam, I., J.P.J. Versteeg, and M. de Vlieger. 1965. Nine years toxicity control in insecticide plants. Arch. Environ. Health 10:441-448.

Jenkins, R.B., and J.F. Toole. 1964. Polyneuropathy following exposure to insecticides. Arch. Intern. Med. 113:691-695.

Jindal, H.R. 1968. Bilateral retrobulbar neuritis due to insecticides. Postgrad Med. J. 44:341-342.

Kay, K. 1977. Polybrominated biphenyls (PBB) environmental contamination in Michigan, 1973-1976. Environ. Res. 13:74-93.

Kazantizis, G., A.I.G. McLaughlin, and P.F. Prior. 1964. Poisoning in industrial workers by the insecticide, aldrin. Br. J. Ind. Med. 21:46-48.

Lester, R., L. Hammaker, and R. Schmid. 1962. A new therapeutic approach to unconjugated hyperbilirubinaemia. Lancet 2:1257.

Mackerras, I.M., and R.F.K. West. 1946. DDT poisoning in man. Med. J. Aust. 1:400-401.

Patel, T.B., and V.N. Rao. 1957. Dieldrin poisoning in man. Br. Med. J. 1:919-921.

Pittman, K., M. Weiner, and D.H. Treble. 1976. Mirex kinetics in the Rhesus monkey, Drug Metab. Dispos. 4:288-295.

Reuber, M.D. 1978. Carcinogenicity of Kepone. J. Toxicol. Environ. Health. Perspect. 4:895-911.

Skalsky, H.L., M.W. Fariss, R.V. Blanke, and P.S. Guzelian. 1979. The role of plasma proteins in the transport and distribution of chlordecone (Kepone) and other polyhalogenated hydrocarbons. Ann. N.Y. Acad. Sci. 320:231-237.

Spiotta, E.J. 1951. Aldrin poisoning in man. Arch. Ind. Hyg. Occup. Med. 4:560-566.

Taylor, J.R., J.B. Selhorst, S.A. Houff, and J. Martinez. 1978. Chlordecone intoxication in man. I. Clinical observations. Neurology 28:626-630.

Wigglesworth, V.B. 1945. A case of DDT poisoning in man. Br. Med. J. 1:517.

Yoshimura, H., and H. Yoshihara. 1975. A novel route of excretion of 2,4,3',4'tetrachlorobiphenyl in rats. Bull. Environ. Contam. Toxicol. 13: 681-688.

DISCUSSION

DR. MILLER: There were two lessons I learned from your presentation. First, by understanding pathogenesis, one may hit on a novel idea for treatment. Second, pollutants can be carried on the clothes of the worker into their homes, where they can affect or at least get into the blood of the family. But you did not mention how Kepone was first recognized as harmful to workers.

DR. GUZELIAN: The workers in this factory believed that there was some adverse health effect related to Kepone. They ate in the room where the dust was packaged and developed some clinical manifestations -- generally tremors, called the "Kepone shakes." The workers repeatedly asked the company medical directors whether or not the chemical was harmful. They were told it was not.

Several of the workers sought medical advice in the community. One in particular did not receive a careful review of his work history, and his complaints were dismissed as effects of anxiety. He was treated with tranquilizers. The cause was not identified until a general practitioner took a careful work history and forwarded blood samples to the Centers for Disease Control (CDC). These samples contained high amounts of chlordecone, which led to the eventual descent on the town by Federal and State health officials.

DR. MILLER: The point I wanted to bring out was that an alert clinician referred the specimens to the CDC.

PROF. GARATTINI: In the preliminary screening, did you find any other agent that was able to bind chlordecone? According to some studies we conducted in mice, cholestyramine is not effective in ridding the body of TCDD (2, 3, 7, 8-tetrachlorodibenzodioxin).

DR. GUZELIAN: We have some other agents, including XAD-2, which seemed almost as effective as cholestyramine. We have not studied mice, but there is an important species difference between humans and rats. I think it would be important to test cholestyramine in higher animals rather than in rodents because the latter do not have very prominent inhibition of their nonbiliary mechanism for handling these substances.

Cholestyramine probably does not act solely by binding Kepone, as I originally thought, also indirectly by binding bile salts and releasing inhibition of the nonbiliary mechanism. Bile salts may be important in altering the disposition of chemicals across the mucosal barrier of the gut wall. This area needs to be investigated thoroughly. Despite negative results in rodents, I would not dismiss this approach to therapy for any of these compounds.

DBCP

Donald Whorton[1]

Dibromochloropropane (DBCP) was a widely used nematocide until it was discovered to cause adverse effects on testicular function among formulation and production workers. When formulation workers at a California pesticide plant noticed infertility problems, they joined together to investigate. Studies at this plant showed a strong association between altered testicular function (azoospermia and oligospermia) and workplace exposure to DBCP. Subsequently, studies at other U.S. and Israeli DBCP production facilities have shown similar results. Followup studies have shown the effects to be reversible when testicular function is not severely affected.

Dibromochloropropane (DBCP) is a liquid nematocide used widely in the United States since the mid-1950's on such crops as citrus fruits, grapes, peaches, pineapples, tomatoes, and soybeans. In Central America and Israel, it is used on bananas. Thus, it is applied both to annual and perennial plants, and harms neither.

The toxicity of DBCP has been recorded in the open scientific literature. In 1961, it was shown to be a mild irritant and an hepatic and renal toxin and to produce testicular atrophy in laboratory animals. (Torkelson *et al.*, 1961). In 1973, it was reported to be carcinogenic in animals (Olson *et al.*, 1973) and in 1975, it was also determined to be a mutagen *in vitro* (Rosenkranz, 1975). Finally, in 1977, it was shown to cause infertility and sterility in humans (Whorton *et al.*, 1977).

---

[1] Department of Biomedical and Environmental Health Sciences, School of Public Health, University of Califorina, Berkeley.

The occupational population at risk includes both formulators and applicators of the compound. The fertility problem was first noticed at a manufacturing plant in central California, which produced DBCP, ammonia, and fertilizers (Whorton *et al.*, 1977). The pesticide formulation area of the plant was small, employing less than 15% of the company's work force. Men in that part of the plant were young, and many wanted to have children. However, the investigators noted that men working in that section of the plant ceased to have children.

After considerable discussion among themselves, five of the employees decided to conduct their own investigation and took semen samples to a local laboratory for analysis. The laboratory would not release the results directly to the men, and I became involved, receiving and subsequently verifying the abnormal findings.

The remainder of the workers in that area of the plant, 36 men, were then tested. Of these, 11 had had vasectomies; the 25 remaining men provided semen samples for analysis. These 25 were divided into three groups, according to length of exposure, which was determined by length of employment in that section of the plant. Eleven men in Group A had the longest exposure: an average of 8 years; range, 4-15 years. The 11 men in Group B were either exposed very briefly (0.08 years) or exposed to low concentrations. Group C consisted of three men whose exposure fell between Groups A and B.

The mean sperm counts of these groups were significantly different: Group A was 200,000, as compared to 93,000,000 in Group B (Table 1). In addition, 9 of the 11 men in Group A were azoospermic and had significantly increased levels of follicle-stimulating and luteinizing hormones. There were no other group findings such as physical symptoms or laboratory results, other than abnormalities of testicular function. The three men in Group C had been exposed for approximately 1 year and had sperm counts ranging between 10-30 million.

Because of the range of findings in the exposed workers, the rest of the employees at the plant were studied under contract to the National Institute of Occupation Safety and Health. A control group of 35 people with no exposure was developed. The duration of exposure to DBCP was estimated for each of 90 men, but could not be estimated for 17 others known to have been exposed. The following ratios were derived. For controls, the ratio of those with a sperm count higher than 20 million/ml to those with a sperm count less than 20 million/ml was 34 to 1. For the exposed groups, the ratio decreased with length of exposure to DBCP -- from 11 to 1 for those exposurd 1-6 months to 0.31 to 1 for those exposed more than 42 months (Table 2).

There were six similar studies in the United States and one Israeli report during 1977-78 (Table 3). All were cross-sectional epidemiologic clinical evaluations, and have been reported in literature or at some scientific symposium or forum. All study subjects had some exposure to DBCP, although exposure was not uniform in duration or concentration. Of 485 men examined, 14.6% were azoospermic and

TABLE 1

Comparison of Nonvasectomized DBCP Workers With Very Low (Group A) and Normal (Group B) Sperm Counts[a]

Group	No. of Subjects	Age, Years	Exposure, Years	Sperm Count $\times 10^6$/ml	Follicle Stimulating Hormone, mIu[b]ml	Luteinizing Hormone, mIu/ml	Testosterone ng/dl
A	11[c]	32.7 ± 1.6[d]	8.0 ± 1.2[e]	0.2 ± 0.10[e]	11.3 ± 1.8[e]	28.4 ± 3.3[d]	459 ± 35
B	11	26.7 ± 1.2[d]	0.08 ± 0.02[e]	93 ± 18[e]	2.6 ± 0.4[e]	14.0 ± 2.8[d]	463 ± 31

---

[a] From Whorton *et al.*, 1977. All results given as mean ± standard error of the mean.

[b] mIu = milli International unit

[c] Nine were azoospermic.

[d] $p < 0.01$

[e] $p < 0.001$

TABLE 2

Ratio of Oligospermia to Normospermia for 126 Nonvasectomized Men Exposed to DBCP for Different Lengths of Time[a]

Exposure	No. of Subjects	Ratio of Subjects with Sperm Counts > 20 million/ml to those with Sperm Counts < 20 million/ml
None (Control group)	35	34
1-6 Months	48	11
7-24 Months	14	2.5
25-42 Months	12	0.5
> 42 Months	17	0.31

---

[a] From Whorton *et al.*, 1979.

TABLE 3

Summary of DBCP Studies 1977-78

Study	No. of Subjects Examined[a]	No. of Azoospermic and Oligospermic Men by Sperm Count, Millions/ml		
		Azoospermic (0 count)	Oligospermic 0.1-9	Oligospermic 10-19
Oxy Chem (California)[b]	114	15	8	12
Shell (Colorado)[c]	64	5	2	7
Shell (Alabama)[c]	71	1	6	5
Dow (Arkansas)[d]	86	30	(17)[e]	(3)
California Applicators[f]	74	6	8	(7)
Israel[g]	23	12	6	0
EPA[h] (North and South Carolina and Texas)	53	2	12	8
Totals	485	71	59	42
Percent		14.6	12.2	8.7

---

[a] Variable exposures.
[b] Whorton *et al.*, 1977, 1979.
[c] Lipschultz *et al.*, 1980.
[d] Scharnweber, 1982; Glass *et al.*, 1979.
[e] Numbers in parentheses extrapolated by me from data presented by original author
[f] Glass *et al.*, 1979.
[g] Potasnik *et al.*, 1979.
[h] Sandifer *et al.*, 1979.

20.9% were oligospermic. Thus, approximately 35% of the men studied exhibited a significant biologic effect resulting from their DBCP exposure.

At the central California plant, 12 men were azoospermic in 1977 and remained so in 1978. Of the men who were oligospermic in 1977, six of nine were normal (> 20 million sperm per milliliter) in 1978, (Table 4) (Unpublished follow-up data collected by me in 1979 and 1980 show the 12 azoospermic men in 1977 and 1978 to have remained azoospermic.).

Biopsies from testicles from these exposed California men were also studied. In an individual exposed less than 3 months, the seminiferous tubules showed marked cellularity and active spermatogenesis. However, a biopsy from an azoospermic individual with 10 years' exposure showed seminiferous tubules that were somewhat collapsed and devoid of cellularity. The cells present were Sertoli's cells. There was no evidence of spermatogenesis. The insterstitial stroma had increased minimally, probably due to the collapse of the seminiferous tubules rather than to fibrosis. There was no evidence of inflammation; the spermatogenic cells seemed simply to have disappeared. Thus, the pathological examination showed no inflammation, no fibrosis, a Sertoli-cell only appearance, and normal interstitial cells, both under light and electron microscopy.

Several questions remain unanswered regarding DBCP exposure. Those men who were less exposed and less affected by the DBCP resumed spermatogenesis. However, no study has been performed of their

TABLE 4

1977 vs. 1978 Sperm Count of 21 Men Exposed to DBCP Longer Than 30 Days[a]

Sperm Count, million/ml	No. of Men, by year	
	1977	1978
0	12	12
0-9	4	1
10-19	5	2
> 20	0	6
Total	21	21

---

[a] From Whorton and Milby, 1980.

Also unaddressed is the possible carcinogenic effect of DBCP in humans. DBCP is known to be carcinogenic in animals and mutagenic in *in vitro* systems. Thus, those men with families and no longer concerned with future parenthood are especially concerned with the carcinogenic aspects of their exposure.

The third problem is the unresolved question of the chemical's effect on women; none of the studies to date has included enough women to evaluate.

The last concern is the effect of low concentrations of DBCP in drinking water. The presence of the chemical is currently an important issue in California, because approximately one-third of the water supply in the central valley comes from deep wells contaminated with varying amounts, ranging from parts per trillion to 5-10 parts per billion.

The last point is a statement rather than a question. Observant and thoughtful workers are responsible for the discovery of the fertility problems caused by DBCP exposure.

REFERENCES

Glass, R.L., R.N. Lyness, D.C. Mengle, et al. 1979. Sperm count depression in pesticide applicators exposed to dibromochloropropane. Am. J. Epidemiol. 3:346-351.

Lipschultz, L.I., C.E. Ross, M.D. Whorton, et al. 1980. Dibromochloropropane (DBCP) and its effects on testicular function in man. J. Urol. 124:464-468.

Olson, W.A., R.T. Habermann, E.K. Weisburger, J.M. Ward, and J.H. Weisburger. 1973. Induction of stomach cancer in rats and mice by halogenated aliphatic fumigants. J. Natl. Cancer Inst. 51:1993-1997.

Potasnik, G., N. Ben-Aderet, R. Israeli, et al. 1979. Effect of 1,2-dibromo-3-chloropropane on human testicular function. Israeli J. Med. Sci. 15:438-442.

Rosenkranz, H.S. 1975. Genetic activity in 1,2-dibromo-3-chloropropane, a widely-used fumigant. Bull. Environ. Contam. Toxicol. 14:8-12.

Sandifer, S.H., R.I. Wilkins, C.B. Loadholt, L.G. Lane, and J.C. Eldridge. 1979. Spermatogenesis in agricultural workers exposed to dibromochloropropane (DBCP). Bull. Environ. Contam. Toxicol. 23:703-710.

Scharnweber, H.C. 1982. The Dow experience. Pp. 30-42 in C. Meyer, M. Curry, and J. Lybarger, eds. Proceedings of NIOSH Conference on Dibromochloropane, Cincinnati, Ohio, October 19-20, 1977. National Institute for Occupational Safety and Health, Washington, D.C.

Torkelson, T.R., S.E. Sadek, V.K. Rowe, J.K. Kodama, H.H. Anderson, G.S. Loquvan, and C.H. Hine. 1961. Toxicological investigations of 1,2-dibromo-3-chloropropane. Toxicol. Appl. Pharmacol. 3:545-559.

Whorton, M.D., R.M. Krauss, S. Marshall, and T.H. Milby. 1977. Chemical induced fertility among employees in a pesticide formulation facility. Lancet 2:1259-1261.

Whorton, M.D., T.H. Milby, R.M. Krauss, and H.A. Stubbs. 1979. Testicular function in DBCP-exposed pesticide workers. J. Occup. Med. 21:161-166.

Whorton, M.D., and T. H. Milby. 1980. Recovery of testicular function among DBCP workers. J. Occup. Med. 22:177-179.

DISCUSSION

DR. REHDER: With experimental mutagenic substances, we know that, in many cases, after exposure there can be recovery if some spermatogonia are left, but you showed tubules without spermatogonia. Have you any experience with these workers after the exposure has ceased? Is there new spermatogenesis? What do testicular histologies look like in between those normal and those very excessive alterations? Where does the damage start -- in the spermatogonia -- or is there a spermatocytic arrest before?

DR. WHORTON: We did study men who had a range of exposures and a range of sperm counts. It appears that the primary spermatogonia are the target cells for direct toxic effect. Testes of lesser injury had foci of spermatogenesis that looked normal, intermixed with foci of large areas containing only Sertoli cells. So, there did not appear to be an interference with the spermatogenic process once that process started. The primary spermatogonia appeared to be the target cell.

As far as recovery goes, several men who have not had any DBCP exposure for more than 10 years are still azoospermic. Apparently, the condition is permanent. Probably most of the men, at least in this group that I have been following who are azoospermic, are probably going to remain azoospermic because of their long exposure.

Some individuals at Dow, in Arkansas, were azoospermic, but have shown some evidence of recovery. However, other men at the same place have not recovered, at this point.

Now, if you use animal or human radiation or chemotherapeutic data as a guide, you may have to wait 3 to 6 years before deciding that an azoospermic individual will remain so permanently. There definitely is a dose-response relationship; what it is -- how much, I don't know.

PROF. DARDANONI: Is there any suggestion of nonoccupational exposure of general population to this compound?

DR. WHORTON: Yes, in California, nonoccupational exposure comes from drinking water; the route of entry is ingestion. Usual workplace route of entry is either by inhalation or skin absorption. How much difference this type of exposure makes is not known.

DBCP was a restricted chemical, used primarily in agriculture, not in the home garden.

DR. DEROSIER: In what concentration does the compound appear in drinking water?

DR. WHORTON: The highest I have seen has been about 5 to 10 ppb. Mostly it has been around 1 to 2 ppb.

DR. MILLER: Is there any evidence that the workers brought the compound home on their clothing and affected the family?

DR. WHORTON: At the plant with which I am familiar, they had special workclothes, took showers, and had to change clothes to come home. Such precautions do not occur elsewhere.

DR. MILLER: On looking at the pathology in the testes, what kind of testicular cancer might develop? Can you predict the cell type?

DR. WHORTON: No, I cannot. For testicular cancer, the annual incidence rate is something like three in 100,000. Half of the cancers are seminomas, and the other half belong to six or seven different groups. Cancer of the testes histologically is defined according to the predominant cell type, because most of them tend to be teratomas.

DR. NELSON: Is there any evidence in animal studies, dominant lethal, or multigenerational studies, of mammalian mutagenicity? When conception can occur, is there any evidence that the father transfers the compound to offspring?

DR. WHORTON: At this time, I am not familiar with that research. Incidentally, there was almost no research on this compound until 1977.

DR. SUSKIND: First, would you comment on the recovery rate of the oligospermic person? Second, is it true that one case of testicular cancer has been reported? Third, what evidence is there that that cancer may have resulted from heavy exposure to DBCP? Fourth, what is the accuracy of the new film, "The Song of the Canary?"

DR. WHORTON: For the most part, oligospermic men recover within the first year after exposure. I am following some severely oligospermic individuals who were not exposed for a long period but they may not recover. Some oligospermic persons have had an increase in fertility in the first year and a half after cessation of exposure.

One individual in Arkansas developed testicular cancer, an embryonal cell carcinoma. He had worked with DBCP for 2 years and with ethylene dibromide for about 8 years. Apparently, he also had had a history of testicular trauma as a youth. So the cause of the cancer is unknown. As far as I know, that is the only case.

The problem has been the lack of a large enough population to study DBCP's possible other effects. One lone factory population is not large enough for study. And the National Institute for Occupational Safety and Health is still in the process of setting up a registry. As to "The Song of the Canary," a completely, scientifically accurate film puts everybody to sleep. Overall, I think it is a fair film.

DR. MOORE: Are researchers also studying applicators or fieldworkers?

DR. WHORTON: OxyChem has conducted the only study on formulators. Shell, Denver; Shell, Alabama; and Dow, Arkansas were done on primary manufacturers. The California study on applicators was done by the Centers for Disease Control and the California Department of Health. The Environmental Protection Agency study was done by investigators from the University of South Carolina, also on applicators in the south. The Israeli study was done on manufacturers.

DR. MURPHY: Can you calculate a dosage level that affects workers?

DR. WHORTON: We have attempted to relate dosage. The problem in the California study, the one at OxyChem, is that the company has no worker records, so, we really have had to rely on people's memories for determining how long they worked.

During the 1960s, DBCP was put in fertilizer, and workers in that period say exposure levels were very high compared to what they are now.

DR. HOOPER: Has anyone studied sperm morphology in these workers? Is there any age distribution in these workers? Are older men, for instance, more susceptible to azoospermia?

DR. WHORTON: Only in the fact that older men worked around the chemical longer, but we saw no real age effect.

As far as sperm abnormalities, there is one paper in the literature by Kapp, who used double Y bodies to look at the Arkansas workers. There was a statistically significant increase in double Y bodies among individuals who had been exposed to DBCP.

Increased Lead Absorption with Anemia and Slowed Nerve Conduction in Children Near a Lead Smelter

Philip J. Landrigan[1]

Studies to evaluate the prevalence, sources, and health consequences of lead absorption were conducted among children living near a primary lead smelter. Lead levels in air, soil, and dust were highest at the smelter and decreased with distance. Nearly 100% of 1- to 9-year-old children living within 1.6 km had lead levels in blood ≥ 40 μg/dl, indicating increased absorption, and 22% had levels ≥ 80 μg/dl. The prevalence of lead levels ≥ 40 μg/dl decreased with distance; 72 km from the smelter, the concentration was 1%. Erythrocyte protoporphyrin levels increased with lead levels in blood: 17% of children with lead levels of ≥ 80 μg/dl were anemic. There was no overt neurologic toxicity. In 202 5- to 9-year-old children, a significant negative correlation was found between blood lead levels and motor nerve conduction velocity ($r = -0.38$, $p < 0.02$).

Lead smelters and refineries in the United States discharge more than 900 metric tons of particulate lead into the air each year (National Academy of Sciences, 1972). Even though this is only a tiny fraction of the 91,000 to 136,000 metric tons emitted annually into the atmosphere, most of which comes from automobile emissions, smelters are fixed sources of emission and thus serve as useful models for studying human exposure to lead in the environment.

In April 1974, an alert pediatrician in northern Idaho saw two siblings, 2 and 3 years old, who complained of abdominal pain, otitis media, and diarrhea. The physician realized that the children lived within 3 km of a large lead smelting plant, and he therefore took x-rays of their knees. The x-rays showed characteristic lead lines in the distal femurs and proximal tibias. The doctor then measured lead levels in the blood of the children and found concentrations of

---

[1] Division of Surveillance, Hazard Evaluations and Field Studies, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

68 and 89 μg/dl. At this point, he contacted public health authorities, who launched a major epidemiologic investigation.

The study showed that lead concentrations in the atmosphere near the smelter had increased steadily through the early 1970's. Mean monthly lead emissions from 1955 to 1964 were 7.3 metric tons/month. Then, from 1965 through 1973, they increased to 10 metric tons/month, and, during 1974, rose abruptly to 32 metric tons/month following a fire, which caused unrepaired damage to the plant's main filtration facility. The plant ran for a year, without filtration, discharging at least 1,800 metric tons of finely particulate lead into the atmosphere.

Concentrations of lead in the air were highest immediately adjacent to the smelter, and decreased with distance from the plant (Yankel *et al.*, 1977). Contamination was virtually nonexistent 55 km away.

For the epidemiologic study (Landrigan *et al.*, 1976), the valley surrounding the smelter was divided into a series of concentric circles. The first had a radius of 1.6 km; the second, a radius of 4 km. Additional circles were drawn with increasing distances from the plant. Lead levels were measured in the blood of several hundred children living within each circle. Numerous environmental samples (air, dust, soil, food, water, and paint) were also evaluated to measure children's exposure from these sources.

A striking pattern was found in the lead levels in the children's blood. The closer children lived to the plant, the more lead in their blood. Within 1.6 km, 98% of almost 200 children had lead concentrations higher than 40 μg/dl. In fact, 20% of the children had concentrations higher than 80 μg/dl, the level the Centers for Disease Control (1978) consider to signal a medical emergency. Those 30 children were, for the most part, referred for intravenous chelation therapy with edetic acid (EDTA).

At 4 km from the plant, 75% of the children had evidence of increased lead absorption. The lead concentration decreased to 30% farther from the plant, then to 10% in more distant circles. At 72 km from the smelter, in a rural district completely removed from plant emissions, only 1% (one child) had evidence of increased lead absorption. Interestingly, that child's father was a painter, and the child may have been exposed to the parent's paint rather than to the smelter.

Other analyses revealed that lead concentrations in children's blood also correlated rather closely with the children's exposure to lead in air; the correlation coefficient between lead in air and blood was 0.72 in a multiple regression analysis. There was also a fairly close correlation coefficient (0.59) between lead levels in soil and blood (Yankel et al., 1977).

An examination of results from epidemiologic studies in relation to various demographic factors showed a correlation between lead concentrations in blood and socioeconomic indicators. Children of higher socioeconomic status had lower lead concentrations in blood than did children of lower socioeconomic status living in the area.

The children of smelter workers, as a group, generally had higher blood concentrations of lead than did other children in the same geographic area. This is another indication that workers carry noxious substances home, thereby exposing their families to secondary contamination.

After the epidemiologic studies, pathophysiologic surveys were conducted to determine whether or not the increased absorption of lead had produced any harmful effects in the exposed children. Studies using hematocrit levels as an index of anemia showed that children in the towns near the smelter were well nourished and that anemia was not a common problem. Only 2% of the 1,000 children screened had hematocrit values below 33%. However, there was a striking correlation between the distribution of anemia in relation to lead levels in blood. Nearly 20% of the children with blood levels greater than 80 μg/dl were anemic, and only 1% of the children with lead levels below 80 μg/dl had evidence of anemia.

There is a fairly strong correlation between erythrocyte protoporphyrin (EP) concentrations and levels of lead in the blood (Granick et al., 1972). In Figure 1, the correlation coefficient with blood lead on the horizontal axis and the EP logarithm on the vertical is 0.79, which agrees with findings of other studies of adults and children exposed to lead (Granick et al., 1972; Piomelli, 1980). The EP elevation is, in all likelihood, a precursor of the anemia observed in the children with the highest lead concentrations in blood.

Finally, peripheral nerve motor conduction velocity in relation to lead exposure was studied. The 5- to 9-year-old children with lead levels greater than 40 μg of lead were compared to a similar

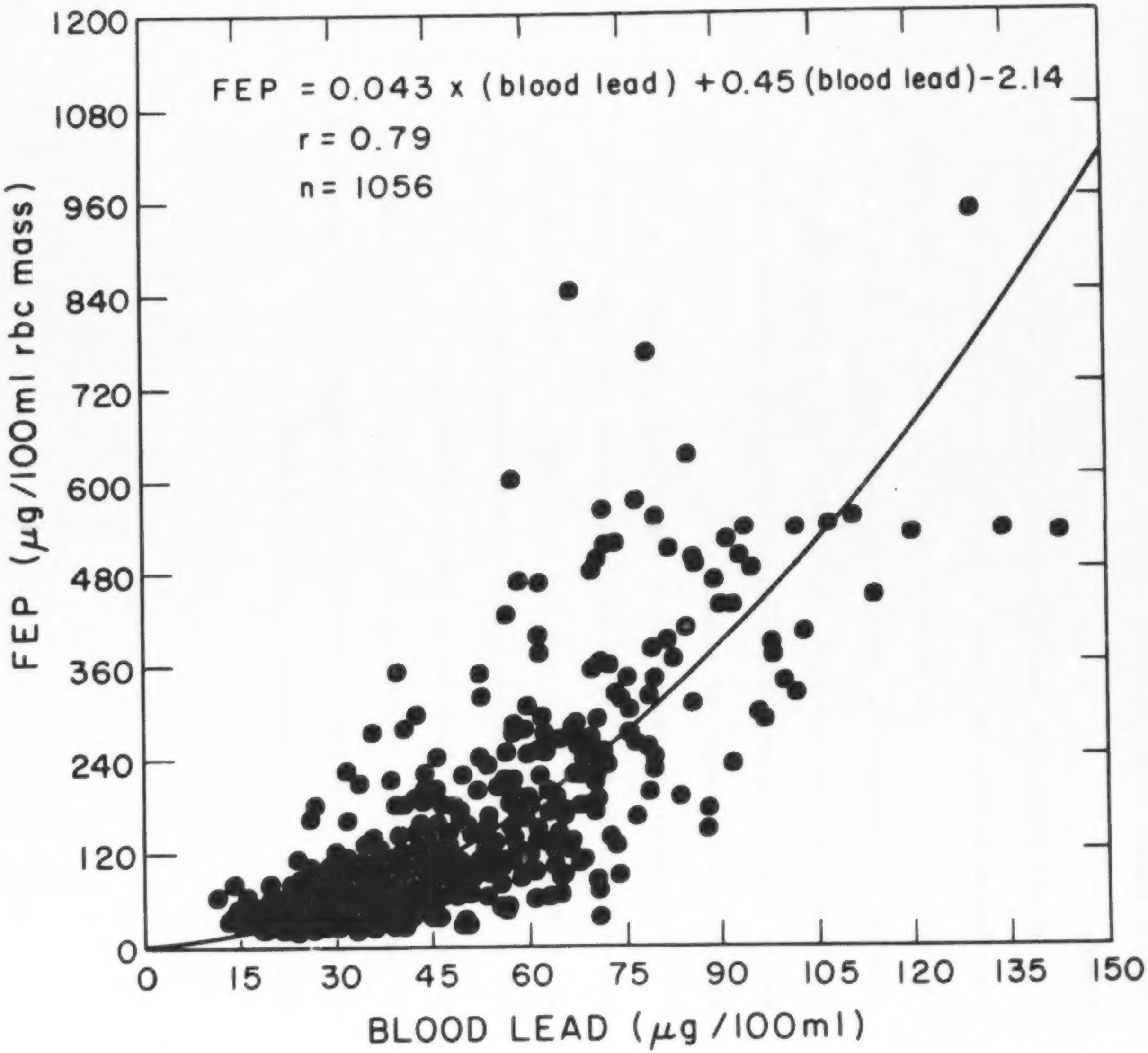


FIGURE 1. Free erythrocyte protoporphyrin (FEP) versus blood lead levels in children 1 to 9 years old in Shoshone County, Idaho, in 1974.

group of children with lower concentrations of lead in blood. Each child first received a very careful neurologic examination: those found to have obvious, preexisting neurologic lesions were excluded from further study. Some children, for example, had microcephaly; others, anoxic birth injuries; and one had been treated for a brain tumor.

In 202 apparently healthy children there was an interesting correlation between the level of lead in blood and motor nerve conduction velocity. As the lead level increased, the conduction velocity decreased. The correlation coefficient there, in the negative direction, was 0.39. Making the same analysis with EP as the independent variable provided essentially the same result with the same strength of association.

In summary, increased absorption of lead, defined as a lead concentration in blood of 40 μg/dl or higher, was highly prevalent among children who lived near the Idaho smelter. The finding that 98% of the children within 1.6 km of the smelter had increased lead concentrations in blood is the highest prevalence that has been recorded. Finally, there were close correlations between blood and environmental lead levels and between lead absorption and anemia, EP elevation, and the decrease in nerve conduction velocity.

REFERENCES

Center for Disease Control. 1978. Preventing Lead Poisoning in Young Children. Center for Disease Control, Atlanta.

Granick, S., S. Sassa, and J. L. Granick. 1972. Assays for porphyrins, delta aminolevulinic acid dehydratase, and prophyrinogen synthetase in microliter samples of whole blood: Applications to metabolic defects involving the heme pathway. Proc. Natl. Acad. Sci. USA 69:2381.

Landrigan, P. J., E. L. Baker, R. G. Feldman, et al. 1976. Increased lead absorption with anemia and slowed nerve conduction in children near a lead smelter. J. Pediatr. 89:904-910.

National Academy of Sciences. 1972. Lead: Airborne Lead in Perspective. National Academy of Sciences, Washington, D.C. 330 pp.

Piomelli, S. 1980. The effects of low-level lead exposure on heme metabolism. Pp. 67-74 in H. Needleman, ed. Low Level Lead Exposure: The Clinical Implications of Current Research. Raven, New York.

Yankel, A. J., I. H. von Lindern, and S. D. Walter. 1977. The Silver Valley lead study: The relationship between childhood blood lead levels and environmental exposure. J. Air Pollut. Control Assoc. 27:763-767.

DISCUSSION

DR. MILLER: How do the health effects you observed compare with the dogma that encephalopathy is the only effect of lead in children, and that lesser effects are not seen?

DR. LANDRIGAN: That point of view prevailed during the 1940's and perhaps into the 1950's. However, as more sensitive hematologic and diagnostic techniques have been developed, it has become evident that there is a wide spectrum of damage induced by lead in children, ranging from minimal slowing of nerve conduction velocity and minimal learning deficits to encephalopathy (Needleman and Landrigan, 1981). This continuum of toxicity relates to increasing exposure to lead.

DR. MILLER: Do you wish to comment about using children's teeth to measure lead?

DR. LANDRIGAN: Studying teeth has turned out to be a very exciting way to measure a child's exposure to lead. Whereas the lead level in blood reflects only very recent lead exposure, perhaps only that during the previous 3 days or in the past week, and the EP level reflects exposure over the past 2 or 3 months, the lead level in a deciduous tooth presents a cumulative exposure record of 7 or 8 years' duration. This technique enables us to correlate a child's longitudinal neurologic and psychomotor development with past lead exposure, perhaps even to exposure before birth. The recent studies of Needleman *et al*. in Boston showed some very good correlations between children's lead levels in teeth and the occurrence in those children of various subtle learning disabilities and psychomotor problems (Needleman _et_ _al_., 1979).

DR. MILLER: Can you comment on the famous Glasgow lead study based on blood specimens collected for PKU (phenylketonuria) screening? The samples were filed away for years, then retrieved and analyzed for lead to show the blood concentrations of mentally retarded children as compared with those of normal children at birth (Beattie _et_ _al_., 1975).

DR. LANDRIGAN: The retarded children showed higher blood lead levels than did the normal children. The presumed source of their exposure was the very heavily contaminated acidic water of Glasgow, which was stored in lead cisterns above the children's houses.

DR. GOLDBERG: Were there any followup studies on the children in Idaho to look at the long-term effects of their lead exposure?

DR. LANDRIGAN: No followup studies have been conducted in Idaho. However, followup study of children with lead exposure near a smelter in El Paso, Tex., showed that the level of lead in children's blood decreased considerably over a period of 5 years (Landrigan _et_ _al_., 1975; Morse _et_ _al_., 1979).

PROF. DARDANONI: Since the Idaho smelter lies in a valley, the prevailing wind may diffuse the lead in one direction. Is there any possibility of determining whether "hot spots" -- lead concentrations in the soil -- exist and correlating these findings to different patterns of human exposure?

DR. LANDRIGAN: Yes. We probably did not do that as thoroughly as we would like. The smelter could not be located in a more unfavorable spot. The valley floor is approximately 1,000 meters above the sea and the mountains on either side rise to nearly 2,000 meters, so the smelter sits in the base of a very narrow "V." Furthermore, the valley runs from west to east; thus, the prevailing wind from the Pacific Ocean carries the lead predominantly to the east. As we looked at lead concentrations in the environment and children, we could clearly tell that the pattern of excess contamination extended much farther to the east of the smelter than to the west.

SPEAKER (UNIDENTIFIED): Have you estimated the heavy metal exposures of populations living within several kilometers of the 40 or so lead and copper smelters in the United States.

DR. LANDRIGAN: After we studies lead absorption near the plants in El Paso, Texas (Landrigan _et_ _al_., 1975), and Kellogg, Idaho (Landrigan _et_ _al_., 1976), we conducted a nationwide survey of heavy metal absorption in children living near 21 other ore smelters in the United States (Baker _et_ _al_., 1977). We measured concentrations of lead, cadmium, arsenic, and copper in children living with a 3.2 km radius of each smelter. We also took samples of their blood and urine.

We found that the Kellogg smelter was the most heavily contaminated with lead. The El Paso smelter was second worst. In terms of lead absorption, no other smelters, except for one in Herculaneum, Missouri, caused even a slight problem. We found a problem of arsenic absorption around copper smelters in Tacoma, Washington (Milham and Strong, 1974), and Anacona, Montana. Cadmium absorption was a problem in children living around a zinc smelter in Bartlesville, Oklahoma.

REFERENCES FOR DISCUSSION

Baker, E. L., C. G. Hayes, P. J. Landrigan, J. L. Handke, R. T. Leger, W. J. Housworth, and J. M. Harrington. 1977. A nationwide survey of heavy metal absorption of children living near primary copper, lead, and zinc smelters. Amer. J. Epidemiol. 106:261-273.

Beattie, A. D., M. R. Moore, A. Goldberg, M. J. W. Finlayson, J. R. Graham, E. M. Mackie, J. C. Main, D. A. McLaren, R. W. Murdoch, and G. T. Stewart. 1975. Role of low level lead exposure in the aetiology of mental retardation. Lancet 1:7907-7910.

Landrigan, P. J., S. H. Gehlbach, B. F. Rosenblum, J. M. Shoults, R. M. Candelaria, W. F. Barthel, J. A. Liddle, A. L. Smrek, N. W. Staehling, and J. F. Sanders. 1975. Epidemic lead absorption near an ore smelter: The role of particulate lead. N. Engl. J. Med. 292:123.

Landrigan, P. J., E. L. Baker, R. G. Feldman et al. 1976. Increased lead absorption with anemia and slowed nerve conduction in children near a lead smelter. J. Pediatr. 89:904-910.

Milham, S., and T. Strong. 1974. Human arsenic exposure in relation to a copper smelter. Environ. Res. 7:176-182.

Morse, D. L., P. J. Landrigan, B. F. Rosenblum, J. S. Hubert, and J. Housworth. 1979. El Paso revisited: Epidemiologic follow-up of an environmental lead problem. J. Amer. Assoc. 242:739-741.

Needleman, H. L., and P. J. Landrigan. 1981. The health effects of low level exposure to lead. Am. Rev. Public Health 2:277-298.

Needeleman, H. L., C. G. Gunnoe, A. Leviton, R. R. Reed, H. Peresie, C. Maher, and P. Barrett. 1979. Deficits in psychologic and classroom performance of children with elevated dentine lead levels. N. Engl. Md. 300:584-695.

Methyl Mercury (Japan)

Robert W. Miller[1]

A severe, sometimes fatal, neurologic epidemic occurred in the early 1950's around Minamata Bay in Kyushu, Japan. Humans, fish, birds, and cats were affected, but the cause was not determined for 5 years, when it was traced to industrial contamination of the bay with methyl mercury. Congenital Minamata disease (cerebral palsy) was recognized even later. A smaller, but similar epidemic occurred a few years later in Niigata, Japan. Methyl mercury poisoning from food has come mainly from using the chemical as a fungicide for grain meant to be planted and not eaten. The later effects of methyl mercury exposure after areawide contamination have not yet been well described. M. Harada of Kumamoto University found a unique way to identify previous methyl mercury exposure by chemical analysis of dried umbilical cords kept for decades by Japanese families. He was able to identify high levels of methyl mercury, not only at the time of the epidemic known in the 1950's, but also of one in the late 1930's, which had been unexplained. This experience illustrates the importance of preserving specimens now for future studies of human diseases thought to be environmentally induced.

Mercury, dumped by a plastics factory into Minamata Bay, Japan, in the 1950's, was converted into organic mercury that was ingested by fish, thus entering the food chain. Animals, both domestic and wild, were affected by the mercury contamination, as were the residents of the area. A privately printed book, Minamata Disease, written in English (Study Group of Minamata Disease, 1968), said that because of neurologic disability, fish swam upside down, birds could not fly, and cats went mad. The fishermen sold the best of their catch; they and their families ate the worst. Thus, their own families were particularly affected by the contaminated fish.

---

[1] Clinical Epidemiology Branch, National Cancer Institute, Bethesda, Md.

The severe neurological effects were immediately recognizable in children and adults, but the transplacental effects were not described until 7 years after the epidemic (Matsumoto *et al.*, 1965). Harada (1976) included a 1962 photograph (probably a composite) of 13 children with severe cerebral palsy, attributed to their intrauterine exposure to methyl mercury. Twenty-three cases were described in the first comprehensive reports; the expected number was 1 or 2. The most recent publication (Harada, 1978) reports 40 cases.

Japanese families, by custom, save dried umbilical stumps, and Harada was able to obtain these from a family with six children born between 1953 and 1966, the time in which the bay was contaminated with methyl mercury. He also obtained dried umbilical stumps from a family with six children born between 1927 and 1939. Unexpectedly, he found a higher methyl mercury content in the cords of two children in this family born during the late 1930's. Apparently, an earlier epidemic had occurred, which Nishigaki and Harada (1975) related to the production of vinyl plastics at the same factory that was implicated in the 1950's outbreak. People living near the factory recalled an unexplained outbreak of psychiatric illness at the time. The factory did not produce plastic during the war, but resumed production afterward, at which time the Minamata disease epidemic occurred.

Minamata disease occurred not only in Japan, but also in Guatemala; Alamogordo, N. Mex.; and Iraq, among other places. In these instances, methyl mercury was used as a fungicide on grain meant to be planted, but instead was fed to pigs or used for baking. In Iraq, some 6,000 people (including six transplacental cases) were reported to be poisoned (Bakir *et al.*, 1973).

Thus, methyl mercury traceable to water, grain, fish, and animals has been implicated in neurologic disorders observed in adults and newborns.

What we have seen here is a neurologic disorder traceable to contamination of water, grain, fish, and animals. It produces a transplacental effect. We have learned that preserved specimens of human tissue are uniquely available in Japan, but could be obtained in this country or elsewhere. This experience raises the question of whether to save such specimens as teeth, a spot of dried blood, or part of the placenta as a means for retracing our past for environmental contamination.

REFERENCES

Bakir, F., S.F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N.Y. Al-Rawi, S. Tikriti, H.I. Dhahir, T.W. Clarkson, J.C. Smith, and R.A. Doherty. 1973. Methyl mercury poisoning in Iraq. Science 181:230-241.

Harada, M. 1976. Intrauterine poisoning: Clinical and epidemiological studies and significance of the problem. Bull. Inst. Constitutional Med. (Suppl.), Kumamoto University 25:1-60.

Harada, M. 1978. Congenital Minamata disease: Intrauterine methyl mercury poisoning. Teratology 18:285-288.

Matsumoto, H., G. Koya, and T. Takeuchi. 1965. Fetal Minamata disease: A neuropathological study of two cases of intrauterine intoxication by a methyl mercury compound. J. Neuropath. Exp. Neurol. 24:563-574.

Nishigaki, S., and M. Harada. 1975. Methyl mercury and selenium in umbilical cord of inhabitants of the Minamata area. Nature 258:324-325.

Study Group of Minamata Disease. 1968. P. 338 in Minamata Disease. Kumamoto University, Japan.

Chlorinated Hydrocarbons

David Axelrod[1]

Love Canal is a 6.5-hectare landfill located in the southeast corner of the city of Niagara Falls, N.Y. The Canal takes its name from a waterway that developer William T. Love began to dig there at the end of the last century. From 1933 to 1953, the Hooker Chemical Company used the Canal to dispose of wastes from its Niagara Falls manufacturing complex. After Hooker sold the landfill in 1950 to the local board of education, an elementary school was built and the surrounding area developed into a neighborhood of working class, one-family homes. In the 1970's it was suspected that pesticide residues had leached from the landfill into the nearby Niagara River and thence into Lake Ontario. State and Federal environmental agencies took air and soil samples at the landfill in 1976-77. On the basis of their findings and homeowner complaints about chemical odors and illnesses, the State Health Department investigated and declared a public health emergency in 1978. The Governor decided that 239 families should be permanently relocated away from the landfill site. Hooker has acknowledged depositing almost 20,000 metric tons of chemical wastes in the Canal from 1942 to 1952. More than 200 organic chemical compounds have been identified in soil, sediment, and sludge samples taken from the landfill. Many of these compounds are toxic to humans. Epidemiologic and environmental testing continues in a widening circle around the landfill.

The disposal of hazardous industrial wastes is a problem of immense complexity with both immediate and long-term health implications for society. One prime example of the problem exists at the Love Canal landfill in Niagara Falls, N.Y. (Figure 1).

When I first visited the site in 1978, it was barren and foreboding. Only a single scraggly tree stood on the site. The ground underneath was gummy, and chemical ooze clung to my shoes. The sickening stench of chemicals and oddly colored pools of water were everywhere. When I poked a stick into the soil, a viscous, oily fluid came to the surface. Pools of water stood on the playing fields of the elementary school, which bordered the landfill. Near the site's northern edge, mounds of dry, white powder dotted the surface.

---

[1] New York State Department of Health, Albany, N.Y.

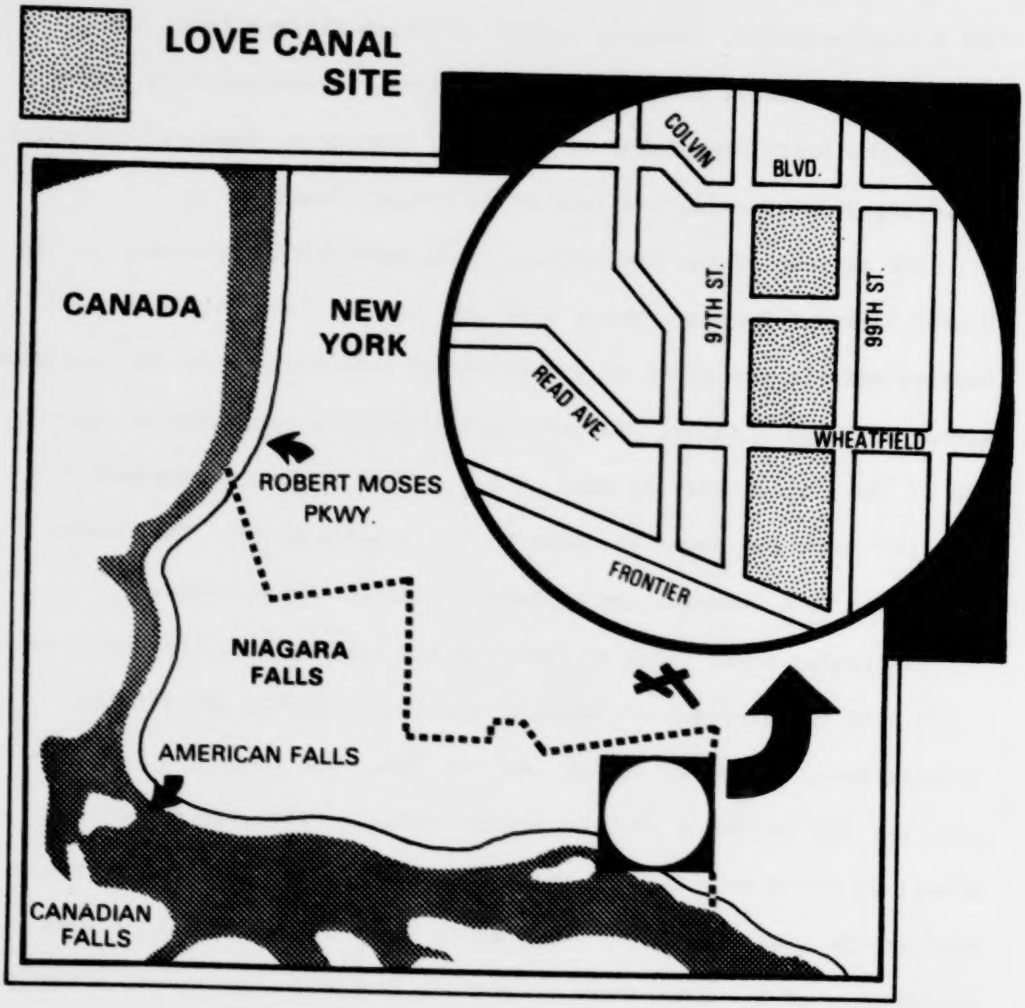

LOVE CANAL SITE
COLVIN
BLVD.
97TH ST.
99TH ST.
READ AVE.
WHEATFIELD
FRONTIER
CANADA
NEW YORK
ROBERT MOSES PKWY.
NIAGARA FALLS
AMERICAN FALLS
CANADIAN FALLS

FIGURE 1. The Love Canal site in Niagara Falls, N.Y.

Someone said this was pure lindane. Fresh motorbike tracks crisscrossed the area and I had visions of toxic dust clouds rising as cyclists raced. Most people know Niagara Falls as a honeymoon resort city of great natural beauty. The landfill is a far cry from the scenic beauty that appears on postcards.

The principal business of the city is the manufacture of chemicals and allied products. Niagara County, in which Niagara Falls lies, harbors an estimated 100 chemical dump sites. According to a survey made by the State Department of Commerce, nine major chemical companies, employing 5,200 people, are located in Niagara County.

The largest of the enterprises, with some 2,000 employees, is the Hooker Chemical Company, which used Love Canal. Love Canal is a 6.5 hectare site in the midst of a residential neighborhood in the southeast sector of Niagara Falls. The site was originally excavated at the end of the 19th century as part of a proposed navigable waterway to link the upper and lower sections of the Niagara River. The river flows into Lake Ontario, the easternmost of the Great Lakes.

Beginning about 1930, Hooker Chemical Company used the abandoned canal site for disposal of chemical wastes, including chlorinated hydrocarbons, processed sludge, and fly ash. The company's records indicate that during a 10-year period ending in 1952, it deposited almost 20,000 metric tons of chemical wastes in the Love Canal area. Whether the canal flowed to the Niagara River is not known. A 1938 aerial view of the area shows no obvious link with the river, although there is a scar in the earth. The landfill was closed in 1953 and Hooker sold the property to the city board of education. Soon thereafter residential development of the surrounding area began, and continued until 1974. In the mid-1950's, a public elementary school was built on land adjacent to the site.

By 1966, the entire landfill had been covered over, and homes were built along the canal. In 1978, 99 houses abutted directly on the waterway, and the first two rings of housing developments around them contained about 230 houses. Approximately, 280 more homes lay beyond these.

The hazard posed by the Love Canal landfill to the residents was noticed by the State Department of Health in 1978. The U.S. Environmental Protection Agency (EPA) and the State Department of Environmental Conservation, had been concerned about potential contamination of the Niagara River and, ultimately, of Lake Ontario. During the course of their investigation, the agencies made a number of soil analyses from properties near the landfill. Afterwards, the Department of Environmental Conservation asked the health department for additional laboratory analyses to determine whether chemicals in the landfill had reached the Niagara River and perhaps accounted for the presence of pesticides in a Lake Ontario fishery.

At that point it was very clear to the Department of Health that the key question was not whether Lake Ontario was contaminated, but whether or not there was a public health problem from the landfill. When the department realized that there was, in fact, a considerable hazard, an order was issued to fence the site and to cover over exposed chemical deposits as a temporary measure until additional studies could be conducted. Infrared photographs showed areas of "coolness," which meant chemical spoliation, and a complete lack of vegetation, even up to the school playground itself. The pesticide lindane, one of the chemicals present, had surfaced and then crumbled off onto the playground and into the street. Water seeped into some of the homes in this area, and also onto the land surface.

Children played on the landfill site and used it as a shortcut to school, even though their shoes, according to anecdote, later fell apart. One youngster reported that the children also used to build treehouses at the site (while the trees were still there), carrying lumber from adjacent areas in the community.

In the spring of 1978, following a period of heavier than normal rain- and snowfalls in the Niagara area, there were many complaints about chemical odors from Love Canal. People living there had, in fact, complained of odors previously, but the odors became much more severe that spring.

EPA studies had identified some 26 chemicals in the area. Subsequently, Department of Health studies showed that about 200 chemicals had been deposited in the landfill. Among these compounds were benzene, toluene, benzoic acid, isomers of dioxin, lindane, trichlorethylene, dibromoethane, benzaldehydes--in effect, most of the chlorinated benzenes and toluene present around a chemical manufacturing plant.

The department is continuing environmental sampling at the site to determine if the levels and types of chemical exposure can be correlated to epidemiologic findings. At present, the department has taken some 3,000 soil samples, from both sides of the canal, for analysis for a variety of different chemicals. The objectives of the environmental and toxicologic studies have been fourfold: (1) to identify the chemical agent or agents responsible for adverse health effects reported by Love Canal residents; (2) to ascertain, if possible, the route of entry of such agents into the human body; (3) to determine the extent and means of chemical migration outward from the landfill proper; and (4) to evaluate the efficacy of the remedial construction taken at the landfill.

From early 1978 and well into 1979, the health department dispatched teams of epidemiologic investigators to the site to determine the extent of adverse health effects. In addition, a nationwide, toll-free telephone hotline was established and publicized through major news media to contact persons who had moved away from the area. About 700 former residents have been reached through this hotline. Within a few weeks in mid-1978, the department had also taken blood samples from some 2,800 people who resided, or had previously resided, in the Love Canal area.

The teams administered a 29-page questionnaire to all residents within a 4-block area of the landfill to obtain detailed information on present and past health status, as well as on social, occupational, and residential histories. The questionnaire covered more than 150 physical complaints or symptoms, ranging from excessive weight loss to headaches and dryness of skin. The Department of Health thus collected a massive amount of health and morbidity data about the people in the area.

Initially, the department selected four health indicators as measures of toxic exposure: miscarriages, birth defects, low birth weight, and liver dysfunction. Adverse pregnancy outcome was selected because the prepartum period is generally considered to be especially susceptible to chemical insult; liver function was studied because many of the chemical compounds identified are known to have hepatic toxicity.

Complete blood counts were performed on 4,386 samples taken from an ultimate study population of 3,919 Love Canal residents. These samples were analyzed for 26 different hematologic and enzymatic parameters.

The department also made an effort to discover the hydrogeology of the area surrounding the landfill. It determined that several "swales" or ditches may have been present in the area. Aerial maps indicate that if water migrated through these ditches over time, the migration pattern apparently has since been greatly disturbed by construction, by the development of storm sewers, and by a variety of other factors. By 1966, aerial overflights indicated only one remaining swale.

Throughout the past 2 years, the department has been trying to discern the normality or abnormality of the epidemiologic evidence. Constantly, data on canal residents have had to be compared with information on population groups that are jarringly inconsistent socially, educationally, economically, and vocationally.

This lack of a control population is the single most important lesson learned from Love Canal. What is desperately needed is a reservoir of scientific information concerning the risks of long-term, low-dose exposure to man-made chemicals. Causal relationships must be established with precision in the face of such confounding health influences as tobacco, occupational exposure, and even genetic factors. It is not inconceivable that significant ill health effects from exposure to the chemicals at Love Canal may not manifest themselves for a generation or longer.

Today, Love Canal has a clay cap, and drainage ditches have been placed to carry the canal's liquids to a facility where they are treated and discharged into the Niagara River.

In summary, much more information is needed to evaluate the toxicity of low-level exposure to chemicals over long periods of time. Furthermore, control populations must be established. Finally, an adequate method is needed to enable a comparison of the kinds of experiences identified at the canal with those in other parts of the country.

DISCUSSION

DR. MILLER: About how many chemicals are really involved at Love Canal?

DR. AXELROD: Hundreds, literally hundreds. A whole variety of chlorinated benzenes, toluenes, phenols, and trichlorophenol in large concentrations suggested that dioxin was also present. It was eventually identified, leaching out from the canal through storm sewers and into the stream-bed in the vicinity.

DR. MILLER: Is chemical dumping limited to Niagara Falls?

DR. AXELROD: No. The Love Canal situation has led to an awareness of a large number of hazardous waste sites throughout the United States. The problem of chemical dumps is a major one for the Nation, and the potential danger needs to be examined further.

SPEAKER (UNIDENTIFIED): How many dumps are there in the United States?

DR. AXELROD: New York State has about 550. A recent EPA report stated that there were thousands.

SPEAKER (UNIDENTIFIED): At Love Canal, was there an odor of organic solvents on wet or rainy days prior to the capping?

DR. AXELROD: On the occasions I visited, there was clearly an odor. The question is whether or not the odor at the site is significantly different from that which exists in adjacent areas. The residents are attuned to the odor and may be able to make that kind of distinction; I could not. Only when I was walking in the area and putting my feet directly into the oozing chemicals could I clearly detect an odor emanating directly from the surface.

DR. LESTER: What comparison group have you been able to find to evaluate some of the health problems?

DR. AXELROD: Generally, the comparison group has come from the area north of Colvin, north of Love Canal.

DR. LESTER: Thus far, how much do you know about chemical migration from the canal area?

DR. AXELROD: We are in the process of analyzing some 3,000 soil samples for evidence of chemical migration. Each sample has to be analyzed for the presence of individual chemicals.

DR. SPENCER: Have you looked for or identified any specific neurologic effects in the population?

DR. AXELROD: We have not examined the population for specific neurologic defects, but the original questionnaire did include a number of items relating to certain identifiable neurologic diseases.

DR. MILLER: Would any purpose be served by studying urine samples to see if they were positive in the Ames test?

DR. AXELROD: We discussed doing Ames tests, but they didn't seem to be the most efficacious method of determining the kinds of exposures. We will continue to look at urine sampling as a way to determine other mechanisms for adverse health effects.

DR. MILLER: Couldn't there be an aggregate effect from the chemicals that might not be determined by identifying individual chemicals?

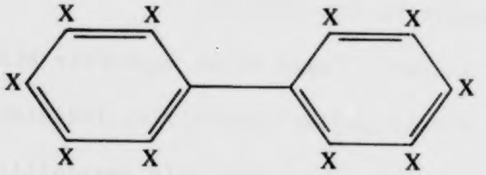
DR. AXELROD: We have discussed this possibility with various individuals involved with the Ames test, and there are conflicting points of view.

Cohort Study of Michigan Residents Exposed to Polybrominated Biphenyls: Epidemiologic and Immunologic Findings

Philip J. Landrigan[1]

Polybrominated biphenyls (PBB's) were dispersed widely in Michigan by a 1973 accident in which PBB's were introduced into cattle feed. Thousands of people were exposed principally from ingestion of contaminated dairy food products. To determine whether PBB exposure has or will cause acute or chronic illness, a prospective cohort study of 4,545 persons has been undertaken. Three exposure groups were analyzed: all persons living on PBB-quarantined farms; persons who had received food directly from such farms; and workers (and their families) engaged in PBB manufacture. Enrollment rates were 95.6%, 95.1%, and 78.0% for each group. Another 725 persons with low-level PBB exposure were also enrolled. All persons were queried about 17 symptons and conditions possibly related to PBB exposure. Venous blood was drawn from 3,639 persons and analyzed for PBB by gas chromatography. Mean serum PBB levels were 26.9 ppb in quarantined farm families, 17.1 ppb in recipients, 43.0 ppb in workers, and 3.4 ppb in the low exposure groups. No associations were found between serum PBB levels and symptom-prevalence rates. To evaluate peripheral lymphocyte function, T and B cell quantitation and *in vitro* responses to three nonspecific mitogens were studied in the 34 persons with the highest PBB levels (mean, 787 ppb), and in 56 with low values (mean, 2.8 ppb). No statistically significant differences were noted in lymphocyte number or function.

In a polybrominated biphenyl (PBB) molecule, there are two 6-carbon rings connected by a carbon-carbon bond:



Bromine is substituted on the rings at any one or more of the 10 positions, thus allowing for an enormous number of possible isomers.

---

[1] Division of Surveillance Hazard Evaluations and Field Studies, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

In the PBB that was the culprit in Michigan, about 80% of the material was hexabromobiphenyl, but other brominated biphenyls, carrying anywhere from one to seven or eight Bromine atoms per molecule, were also detected in the commercial mixture (Matthews et al., 1977).

In the summer of 1973, a small chemical company in St. Louis, Mich. produced two commercial mixes. Nutrimaster contained magnesium oxide and was used to stimulate lactation in dairy cattle. Firemaster was used as a flame retardant, principally in the plastics industry. On one occasion, the company ran out of Nutrimaster bags and packaged several hundred pounds of Firemaster into Nutrimaster bags. The company claims to have written the word Firemaster across the top of the bags, but the written label appeared precisely where a farmer would rip the bag to pour it into cattle feed.

It is estimated that probably several hundred pounds of polybrominated biphenyl were introduced into cattle feed in Michigan that summer. The chemical spread rapidly through the distribution chain and affected thousands and thousands of cattle, which were contaminated and had to be quarantined and destroyed. Other cattle died. The PBB caused devastating illness. Sick cows and calves became emaciated and had trouble walking. A variety of dermatologic disorders appeared, including a bizarre overgrowth of the hoof, which may be the closest manifestation cattle have to chloracne (Jackson and Halbert, 1974).

Many thousands of people were also exposed to PBB by several routes. Workers in the chemical factory that produced the material were exposed directly, probably both by inhalation and inadvertently by ingestion of particulate material. People who lived on contaminated farms were also heavily exposed. The farmers very likely handled feed directly, and the entire farm family ate and drank contaminated meat and milk. Finally, the consumers, the people who bought contaminated dairy products, were also exposed.

To evaluate PBB exposure and health effects in the State of Michigan, the Centers for Disease Control (CDC), in collaboration with the Michigan Department of Public Health, the National Institute for Environmental Health Sciences, the National Cancer Institute, the Food and Drug Administration, and the Environmental Protection Agency (EPA), mounted an enormous cohort evaluation of some 4,000 Michigan residents beginning in 1976 (Landrigan _et al._, 1979). Basically, four categories of Michigan residents were enrolled in the study. First, there were the farm families who had been contaminated. Of 2,200 people contacted, 95% agreed to participate in the study. Second, a number of people were farm product recipients. These people lived next door to or down the road from a contaminated farm. They received their milk or meat directly from a contaminated farm, and there was no dilution of the contaminated product through normal commercial channels.

Third, there was a small group of workers at the chemical plant. The participation of this group and their families was not quite so good. Finally, there was a small group of persons who had participated in a pilot study conducted the previous year by the Michigan Department of Public Health (Humphrey and Hayner, 1975). There were also approximately 600 people, who essentially had no documented exposure to PBB, but who were particularly concerned and wanted to be included in the study.

Everybody enrolled was visited between 1976 and 1977 by trained field investigators, who asked in detail about 17 symptoms that might conceivably be related to PBB exposure (Kuratsune et al., 1972). The list of symptoms included those reported by the subjects and a few related to effects observed in cattle. Fatigue and pains in the joints were the two most prevalent symptoms.

The distribution of serum concentrations of PBB in the various groups was an interesting finding. The range of concentration was very broad in almost every group, yet the medians were very low. Hence, it is evident that there is a log normal distribution of PBB in the serum of these population subgroups.

The highest PBB concentrations in serum were observed in the chemical workers and their families. In fact, looking at just the workers apart from their families, the serum level was even higher. Nonetheless, the family members also had levels much increased above those found in the general population, suggesting a pattern of secondary contamination as workers transport the substance from the workplace home to their families.

After the workers, the next highest serum PBB levels were found in persons living on contaminated farms. Next to this group were the consumers, the recipients of contaminated products. The volunteer participants had almost uniformly low levels.

The study related the serum PBB concentrations to the prevalence of symptoms in the 3,300 persons for whom complete data sets were obtained. The cohort was divided into seven groups, depending on the range of serum PBB concentrations; for example, no concentration, 1 ppb, 2 to 3 ppb, and so on. Of the persons who had no PBB concentration, 46% reported fatigue; of those with 1 ppb, 40% had fatigue, and of those with more than 1 ppb, 27% had fatigue. This finding is essentially an inverse dose-response relationship. A more or less similar pattern was observed for each of the other symptoms.

There are some rather striking differences among groups. For example, the chemical workers had a much lower symptom-prevalence rate than did farmworkers. Prevalence rates within each study group were looked at separately, but the same pattern remained; that is, the highest prevalence of symptoms was observed in the persons with the lowest serum PBB levels.

Thus, there is unequivocal evidence of statewide PBB contamination. That finding is corroborated by the results of other studies (Brilliant et al., 1978; Selikoff and Anderson, 1979). The evidence of the exposure was observed first in chemical workers, then in farmers, then in consumers, as the contamination spread broadly through the food chain. However, nothing was observed to correlate the dose-response relationship to serum PBB levels or to the occurrence of symptoms. There are probably people in Michigan who have symptoms caused by PBB exposure, but those symptoms may never become evident through the epidemiologic technique because the real organic symptoms are masked by nonorganic symptoms.

Analyses of PBB in serum samples taken from several hundred people in 1974 were compared with results from second samples taken in 1977. There was virtually no change in the serum PBB concentrations during that 3-year period. The compound is extremely stable in the body. It is very fat soluble and highly concentrated in fat. A person heavily contaminated with PBB carries a substantial portion of the ingested dose for a lifetime.

In this study, there were also 221 simultaneous paired specimens of blood and adipose tissue. The concentration gradient from adipose tissue to blood was 360 to 1. Dr. Irving Selikoff and his colleagues at Mt. Sinai have also looked at paired adipose and serum tissues of several hundred people. They, too reported an adipose-to-serum PBB gradient of about 350 or 360 to 1 (Anderson et al., 1978). The two sets of data are thus in close agreement and confirm the hypothesis that PBB is highly partitioned in fat.

PBB is also concentrated in the fat portion of breast milk. A survey by the Michigan Department of Public Health showed that 96% of breast milk samples obtained in Michigan's lower peninsula were contaminated with PBB. Analyses of breast milk samples from women in the upper peninsula showed that 43% were contaminated, providing evidence of the geographic transfer of the substance (Brilliant et al., 1978).

In summary, although no definitive evidence of acute or subacute disease has come to light so far in the Michigan population as the result of PBB exposure, considerable concern remains about long-term, possible delayed consequences. Dr. Renate Kimbrough, a toxicologist at the Centers for Disease Control, has developed evidence indicating that PBB causes adenocarcinoma of the liver in rats (Kimbrough *et al.*, 1978). Because Michigan residents will continue to suffer internal biological exposure to PBB for the next several decades, they may have a greater risk of developing cancer. The health agencies that established the Michigan study cohort plan to follow it for the next several decades.

REFERENCES

Anderson, H. A., R. Lilis, I. J. Selikoff, K. D. Rosenman, J. A. Valciukas, and S. Freedman. 1978. Unanticipated prevalence of symptoms among dairy farmers in Michigan and Wisconsin. Environ. Health Perspect. 23:217-226.

Brilliant, L. B., G. Van Amburg, J. Isbister, H. Humphrey, K. Wilcox, J. Eyster, A. W. Bloomer, and H. Price. 1978. Breast milk monitoring to measure Michigan's contamination with polybrominated biphenyls. Lancet 2:643-646.

Humphrey, H. E. B., and N. S. Hayner. 1975. Polybrominated biphenyls: An agricultural incident and its consequences, an epidemiological investigation of human exposure. Presented at the Ninth Annual Conference on Trace Substances in Environmental Health, Columbia, Mo., June 1975.

Jackson, T. F., and F. L. Halbert. 1974. A toxic syndrome associated with the feeding of polybrominated biphenyl-contaminated protein concentrate to dairy cattle. J. Am. Vet. Med. Assoc. 165:427-439.

Kimbrough, R. D., V. W. Burse, and J. A. Liddle. 1978. Persistent liver lesions in rats after a single oral dose of polybrominated biphenyls (Fire Master FF-1) and concomitant PBB tissue levels. Environ. Health Perspect. 23:265-273.

Kuratsune, M., T. Yoshimura, J. Matsuzaka, and A. Yamaguchi. 1972. Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ. Health Perspect. Experimental Issue No. 1, April 1972:119-128.

Landrigan, P. J., D. R. Wilcox, Jr., J. Silva, Jr., H. E. B. Humphrey, C. Kauffman, and C. W. Heath, Jr. 1979. Cohort study of Michigan residents exposed to polybrominated biphenyls: Epidemiologic and immunologic findings. Ann. NY Acad. Sci. 320:284-294.

Matthews, H. B., S. Kato, N. M. Morales, and D. B. Tuey. 1977. Distribution and excretion of 2,4,5,2',4',5',-hexabromobiphenyl, the major component of Firemaster BP-6. J. Toxicol. Environ. Health 3:599-605.

Selikoff, I. J., and H. A. Anderson. 1979. A survey of the general population of Michigan for health effects of PBB exposure. Contract Final Report. Michigan Department of Public Health, Lansing, Mich.

DISCUSSION

DR. MILLER: One of the novel aspects of this experience was using breast milk to analyze the body burden of a chemical that is deposited in fat and not easily removed from it.

DR. LANDRIGAN: Yes, a very strong case can be made for using human breast milk as a device for screening a population's exposure to fat-soluble compounds, whether they are halogenated organics, such as PBB's or polychlorinated biphenyls (Landrigan, 1980), or fat-soluble pesticides (Savage, 1977). Milk is obviously an easy tissue to obtain. It does not require venipuncture, and it is easily transported. Also, because milk fat contains highly concentrated fat-soluble compounds, relatively simple analyses will often suffice for observing trends in population exposure.

DR. MILLER: Are PBB's metabolic activators?

DR. LANDRIGAN: Yes, very much so. They activate microsomal enzymes in the liver (Matthews _et_ _al_., 1978).

DR. MILLER: Would that change a patient's blood level of a chemotherapeutic agent for cancer or of some other drug?

DR. LANDRIGAN: Barbiturate metabolism might, for example, be altered.

DR. NELSON: Can you say anything about the immunologic studies done in relationship to the PBB studies by the Mt. Sinai group? Are there special problems in maintaining surveillance of identified exposed groups for 30 to 35 years?

DR. LANDRIGAN: Two immunologic studies have been conducted in Michigan on persons exposed to PBB's. The first was made by Bekesi *et al.* (1978) from Mt. Sinai Hospital in New York, who examined immune functions in Michigan Residents and compared them with those from people in Wisconsin with no exposure to PBB. The data are curious. Every one of the Michigan residents showed some form, although not the same form, of lymphocytic malfunction. In contrast, virtually none of the Wisconsin residents showed any lymphocytic malfunction. Among the Michigan residents, there was no evidence of a dose-response relationship to serum PBB concentration. Many instances of dysfunction occurred in persons with virtually nondetectable serum PBB concentrations.

The Centers for Disease Control undertook a second study with the University of Michigan and examined 36 persons with very high exposure to PBB (Silva *et al.*, 1979). Their mean serum PBB concentration was 787 ppb, a concentration much higher than that in the group studied by Bekesi *et al.* (1978). CDC also examined a control group of 57 persons with mean serum PBB concentrations of 2.8 ppb. No differences between groups in leukocyte count, in the number of T cells or B cells, or in the *in vitro* responses of lymphocytes to any of three exogenous mitogens were recorded.

DR. MILLER: As to continuing surveillance of exposed groups, there are three keys to success. One, know who was exposed; two, know the body burden or the dose; and three, know an easy, almost effortless way to follow people inexpensively.

Our National Death Index, which has recently been established by the National Center for Health Statistics, will enable easy followup of any person listed on an exposure registry. When a death is reported, we can determine the cause from the death certificate. We will still need to take a census following an acute environmental episode to determine who was exposed, the dose involved, and the followup system needed.

DR. NELSON: How were PBB's finally identified in the feed?

DR. LANDRIGAN: It is a long and complex tale best told in the book Bitter Harvest by Fred Halbert, a chemical engineer turned farmer in Michigan (Halbert and Halbert, 1978). He lost a prize herd of some 3,000 cattle to PBB. The discovery that PBB was the offending agent was due probably more to his efforts than to anything else.

In the beginning, Halbert went to many different laboratories around the country with feed samples. The feed was analyzed by conventional gas chromatographic techniques, and the results were uniformly negative. Fortunately, he persisted and finally went to the U.S. Department of Agriculture field station in Ames, Iowa. Laboratory workers there injected an extract of feed onto the gas chromatograph and left it running over a long lunch break. PBB, which has an unusually long retention time because of its high molecular weight, finally settled during this longer period. When the workers returned, they found a late peak, subsequently identified by mass spectroscopy as PBB.

DR. HUNT: Could you elaborate on the maternal cord-blood relationships?

DR. LANDRIGAN: We looked at the PBB concentrations in the paired maternal and cord blood of 58 maternal-infant pairs. In general, there was a slight concentration gradient favoring the mother, and the average ratio of maternal-to-cord PBB concentration was 7:1. We also had some 32 maternal blood-breast milk pairs, and the ratio of PBB in whole milk to serum was 122:1.

Thus, it is clear that PBB does cross the placenta almost unimpeded. Furthermore, it is concentrated in milk fat and therefore is a continuing hazard to the nursing infant.

DR. HUNT: In terms of quality assurance of analyses of serum and adipose tissue, have you examined the effect of freezing on the stability of the chlorinated hydrocarbons in body tissues over a period of time? This question relates, of course, to whether you can go back to other samples that have been frozen for a long time.

DR. LANDRIGAN: To maintain quality control, an enormous effort has been conducted by the laboratories of the Michigan Department of Public Health and the CDC. Approximately 40% of the total laboratory work in this project has been quality control, not only because PBB is a terribly difficult chemical to analyze, but also because there is a great desire to put these analyses on as close to an absolute standard as possible. The objective is that results obtained 20 years hence will be directly comparable with those obtained today. A freeze-thaw experiment showed no degradation in PBB concentration.

SPEAKER (UNIDENTIFIED): When you discussed the negative dose-response correlation between the prevalence of symptoms and the body burdens of PBB, they were all based on symptoms such as fatigue and joint pain. Were any hard signs used to attempt to generate a dose-response curve?

DR. LANDRIGAN: We did not do physical exams during this study. We were trying to interview, within 1 year, some 4,000 persons widely scattered across the state, and we did not have the personnel to conduct the exams. However, if a person reported seeing a physician during the preceding 2 or 3 years--for any condition--we got permission to contact the physician and collect the medical records on every such visit.

DR. GUZELIAN: People commonly use serum as a way of analyzing exposure, and changes of exposure, in relation to symptoms. Serum may be an inadequate indicator, so I am not surprised that you did not find a dose-response relationship between symptoms frequency and PBB concentration in serum given the incredibly disproportionate distribution of PBB between fat and blood. It is possible in field studies to obtain samples of adipose tissue by using the Garrottson technique, which involves subcutaneous needle aspiration of adipose tissue without anesthesia.

DR. LANDRIGAN: We decided not to use wide-scale fat biopsy because we wanted to have a cohort to look at 30 years from now. We felt fat biopsies might deter people from continuing to participate.

Surprisingly we found a very high correlation coefficient with very little scatter between the PBB concentrations in serum and adipose tissue. It was 0.960. That finding seems to vindicate the use of PBB concentrations in serum as an indicator and gives us assurance that we need not do 4,000 fat biopsies.

REFERENCES FOR DISCUSSION

Bekesi, J. G., J. F. Holland, H. A. Anderson, A. S. Fischbein, W. Rom, M. S. Wolff, and I. J. Selikoff. 1978. Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. Science 199:1207-1209.

Halbert, F., and S. Halbert. 1978. Bitter Harvest: The Investigation of the PBB Contamination -- A Personal Story. William B. Eerdmans Publishing Co., Grand Rapids, Mich.

Landrigan, P. J. 1980. General population exposure to environmental concentrations of halogenated biphenyls. Chapter 9A, pp. 267-286 in R. D. Kimbrough, ed. Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins, and Related Products. Elsevier, Amsterdam.

Matthews, H., G. Fries, A. Gardner, L. Garthoff, J. Goldstein, Y. Ku, and J. Moore. 1978. Metabolism and biochemical toxicity of PCBs and PBBs. Environ. Health Perspect. 24:147-155.

Savage, E. P. 1977. National study to determine levels of chlorinated hydrocarbon insecticides in human milk, 1975-1976, and supplementary report to the National Milk Study, 1975-1976. National Technical Information Services, Accession No. PB284393/As, Springfield, Va.

Silva, J., C. A. Kauffman, D. G. Simon, P. J. Landrigan, H. E. B. Humphrey, C. W. Heath, Jr., K. R. Wilcox, Jr., G. Van Amburg, R. A. Kaslow, A. Ringel, and K. Hoff. 1979. Lymphocyte function in humans exposed to polybrominated biphenyls. J. Reticuloendothelial Soc. 26:341-347.

Case Studies: The Atomic Bomb Casualty Commission

Gilbert W. Beebe[1]

Investigation of the acute effects of the atomic bombs dropped on Hiroshima and Nagasaki in 1945 led to a continuing study of their late effects by the National Academy of Sciences. Under its Committee on Atomic Casualties, the Atomic Bomb Casualty Commission (ABCC) was created to conduct the field investigations in Japan. Although the initial search for genetic effects was well conceived and effectively carried out, the search for somatic effects, initially characterized by ad hoc surveys aimed at particular effects, enjoyed only temporary success. Investigator interest waned, subject participation declined, and serious suggestions were made for closing the operation. The venture was rescued by a reconceptualization of the task along epidemiologic lines. Systematic, multiphasic screening strategies then began to bear fruit in the 1960's as one form of cancer after another was shown to be occurring in excess among heavily exposed survivors and, eventually, to be dose-dependent in quantifiable fashion. The history of the ABCC contains important lessons for those who may plan long-term studies of the health effects of large-scale disasters.

In September 1945, American medical teams joined Japanese already at work in Hiroshima and Nagasaki investigating the acute effects of the disasters (Oughterson and Warren, 1956). On their return to the United States, they proposed a continuing investigation of late effects, and President Truman was persuaded to ask the National Academy of Sciences, as a nonmilitary, nongovernmental organization, to assume responsibility for directing a study of the late effects of the ionizing radiation released by the bombs.

The Academy formed the Advisory Committee on Atomic Casualties (CAC) and a field organization in Japan, the Atomic Bomb Casualty

---

[1] Clinical Epidemiology Branch, National Cancer Institute, Bethesda, Md.

Commission (ABCC). It is noteworthy that there was no epidemiologist among the members of the CAC. In the early years, the ABCC emphasized the building of an organization along conventional, multidepartmental lines and attacked specific hypotheses concerning the nature of the late effects. Investigators were invited to survey survivors for particular end results, but there was no overall research strategy other than the search for particular effects. This worked well for the study of genetic effects, which was well conceived and effectively carried out, but proved to be only temporarily successful with respect to somatic effects. There were some very important findings in the early period (1947-1955)--leukemia (Folley _et al._, 1952), cataracts (Cogan _et al._, 1949), and, after _in utero_ exposure, small heads (Plummer, 1952) and mental retardation (Plummer, 1952)--and an ABCC Radiation Census in Hiroshima and Nagasaki in 1949 paved the way for a nationwide census of atomic-bomb survivors in 1950 at the time of the official Japanese National Census, a census that was to provide a sound sampling base for the program in later years.

By 1952 or 1953, despite the initial accomplishments, the effort was losing momentum and suggestions were made that it be terminated. One consideration was the rapidly mounting cost, which the sponsoring federal agency, the Atomic Energy Commission (AEC), found increasingly burdensome. There also was a marked decline in participation in the clinical examination programs on the part of the Japanese subjects. Once the early effects had been detected, systematic clinical surveys, in which the greatest investment was being made, yielded essentially negative results. The genetic study, although massive in scale, also produced essentially negative results (Neel and Schull, 1956). What had happened, we now know, was that the immediate effects, and leukemia, which has a short minimum latent period, were rather easily and quickly detected, but the solid tumors, which were to become the major findings in later years, were not yet in evidence because of their longer latent periods (Beebe *et al.*, 1978).

The situation became critical in 1955, and Dr. R. Keith Cannan, as Chairman of the Division of Medical Sciences, National Research Council, appointed an *ad hoc* committee, the so-called "Francis Committee", under the chairmanship of Thomas Francis, Jr., the virologist and epidemiologist. The other members of this committee were statisticians: Seymour Jablon, of the Academy staff, and Felix Moore, then at the National Heart Institute. Dr. Cannan asked that this group visit the operation in Japan and recommend action that might be taken in order to fulfill the original mission of ABCC. The Francis Committee prepared a most remarkable document that drew upon the considerable strengths and experiences of the past program, made recommendations for eliminating essential weaknesses, suggested specific methodologic innovations, and visualized an integrated strategy for the conduct of a continuing program (Francis *et al.*, 1959). It provided a conceptual overview within which a variety of research approaches were integrated in a "Unified Study Program." A considerable emphasis was placed on systematic screening of fixed cohorts in different ways and on continuity of effort within permanent research designs.

The committee discovered that much of the work started during the early years remained unfinished. New members of the staff had arrived, started new projects, and completed their fixed tours of duty before their work had been completed. There was a morgue of unfinished projects, tabulations, and manuscripts. To ensure continuity of effort and program in the face of short-term staff assignments was a major objective of the Francis recommendations.

Fortunately, Japan has a nearly unique family registration system that provides for the posting of vital events on a family register in the "home" city. This record makes it possible to trace people for mortality on the basis of their permanent family "home", even if they reside elsewhere. The Francis Committee recommended that this system be used to trace mortality for fixed cohorts defined on the basis of exposure to the bombs. Major samples include survivors' children conceived after the bombs, those exposed postnatally, and those exposed *in utero*. All are critically important groups in the program today (Beebe and Usagawa, 1968).

Almost concurrently with the work of the Francis Committee plans were begun to develop a physical dosimetry program recommended by the AEC. ABCC technicians obtained shielding histories from atomic-bomb survivors. An Oak Ridge National Laboratory group then created dose-distance curves and arranged for experimental work at the

Nevada Test Site in order to develop transmission or attenuation factors associated with environmental shielding (Auxier, 1975). Recently, this group has provided the basis for adjusting essentially external doses to tissue doses for critical organs (Kerr, 1979). These are individual doses with separate components for gamma and neutron radiation.

Initially, the research goal was mainly to identify affects. Now, in contrast, the main emphasis is placed on the measurement of effects in relation to dose, host factors, and other environmental factors. This interest is not merely descriptive; it extends to mechanisms as well (Beebe, 1981).

Since 1955 there have, of course, been changes in the Unified Study Program, changes that have been to some extent responsive to technological change and to advances in radiation biology. Although the Francis Committee proposal for an "epidemiologic detection network," a block-based morbidity reporting system, was abandoned at the outset, all other features were retained: a clinical detection program in which survivors were seen in outpatient clinics, a postmortem detection program, a laboratory detection program, and a death certificate study. These components were unified through common samples or subsamples. Additions to the program include cancer registries, newly designed genetic studies, and, of the utmost importance, a physical dosimetry program. In 1975 an ambitious biochemical genetics study was launched (Neel _et al._, 1980). This study extended earlier genetic studies of pregnancy termination and birth defects (Neel and

Schull, 1956), mortality patterns of the F_1 generation (Neel et al., 1974), and chromosomal aberrations in the F_1 generation that might be indicative of hereditary changes (Awa, 1975).

The atomic-bomb victims themselves have been very cooperative, despite the policy of ABCC not to provide direct medical care except in a few, now-closed diagnostic beds in Hiroshima. There have been no attitude surveys or other social surveys that might give the American investigators a good reading on the feelings of the Japanese victims of the bombings, but the often unfriendly tone of the local Japanese press contrasts sharply with the continued cooperation of the subjects themselves. There is at least one fairly substantial observation: Dr. Robert Lifton (1967), the Yale psychiatrist, suggested that even those who experienced very low doses of ionizing radiation are quite fearful of the future, especially from the standpoint of cancer. This fear exists despite two facts: (1) it has never been possible to demonstrate excess cancer at the low doses experienced by the great majority of the survivors, and (2) the best current estimate is that, from 1950 to 1974, the 285,000 survivors enumerated at the time of the 1950 census may have suffered about 415 deaths as the late effects of exposure to ionizing radiation, an increment of about 1 in 690 survivors, or 0.6 percent of the naturally occurring deaths among them in this period (Beebe et al., 1978). The lack of a program to inform the survivors of their real risks has seemed to me most unfortunate.

Another notable fact has been an almost unrelenting hostility on the part of the local press. Allegations that have been made repeatedly include: the survivors are merely guinea pigs; the aggressors should not be entrusted with the studies; secret data are sent to the United States for use in its own defense; and deaths of atomic-bomb survivors are mainly "A-bomb deaths", attributable to radiation. Although much of the research directly benefited the survivors by providing a diagnostic and referral service, an aggressive program to procure permission to autopsy survivors living in the community during the 1960's, although quite successful and apparently acceptable at first, led eventually to very negative reactions in the press and, in the end, in the community as well.

One should bear in mind that the U.S. staff of the ABCC was for many years under the U.S. Embassy and enjoyed some of the perquisites of diplomatic status. In 1974, when discussions were under way to replace ABCC with the present organization, the Radiation Effects Research Foundation, it became clear that diplomatic privilege was symbolic of foreign control and that the local Japanese, both physicians and community leaders, were anxious to have Japanese control. The Government of Japan either did not want this or did not want to insist on it, and the final agreement called for equality of both funding and top-level management control. The local people also wanted a medical care emphasis that had been lacking throughout the program.

What lessons concerning research strategy can be drawn from the ABCC experience? First, such work must be directed by a comprehensive research strategy devised early. This is not to say that there should be no response to new developments in science, but, rather, that without a firm navigational plan it is very easy for an effort of this kind to wander off course. We saw that ABCC was threatened with closure early in its history. Had it stopped operation in 1955, we would never have learned about the solid tumor effects that became evident only in the 1960's, the first being thyroid cancer in 1962. A research strategy also needs to be enlivened by imagination about techniques that may only later become available, laboratory techniques that might require stored samples of blood or tissue, for example. (These observations and those described below are discussed in greater detail in Beebe, 1979.)

The ABCC experience clearly indicates the tremendous importance of registering the exposed population early, before it scatters widely and becomes infiltrated by people who are looking for compensation or other benefits. In the enumeration process, appropriate identifiers for long-term follow-up must be obtained.

A third point is that the parameters of exposure should be ascertained in fine detail, with emphasis on objective physical measurements and on determinations applicable to the individual if at all possible. This just has to be done early. At ABCC it was possible to obtain exposure histories long after the bombings because the bombing was what Lifton has called a supernatural experience, one that was burned indelibly in the minds of the victims. Even 10 to 15 years after the bombings, it was possible for a technician with a prestrike map to sit down with a survivor and get a pretty accurate indication of where that person was, what he was doing, and what his shielding situation was. But such recall must be highly unusual.

The need for fixed cohorts is also very important, not only to avoid retrospective bias but also to permit absolute risk estimates to be made. And if mortality can serve as the end point in a long-term study, much of the cost of a long-term follow-up study can be saved. I think at ABCC we continued a strong clinical emphasis long after it was most cost-effective.

Staffing patterns are needed that ensure not only competence, but also continuity. ABCC was fortunate in having one director for 15 years, and also benefited from arrangements with key U.S. institutions to assume some responsibility for staffing the major research departments.

An overall prescription for follow-up studies of the long-term effects of disasters would include: (1) Devise an adequate overall strategy early. (2) Register the population of interest as soon as possible, taking care to include all key identifiers on which follow-up may depend. (3) Determine the parameters of exposure in detail, with emphasis on objective physical measurements. (4) Use a cohort approach and plan statistically powerful comparisons to identify and measure effects, either in dose-specific fashion or in exposed vs. unexposed contrasts. (5) Evaluate carefully the potential yield and cost-effectiveness of alternative end points, especially mortality vs.

morbidity and physical defects. (6) Plan staffing patterns to provide not only excellent leadership and scientific performance, but also continuity. (7) In an operation of long duration, endeavor to develop deep local roots.

REFERENCES

Auxier, J. A. 1975. Physical dose estimates for A-bomb survivors--Studies at Oak Ridge, U.S.A. J. Radiat. Res. (Tokyo) (Suppl) 16:1-11.

Awa, A. A. 1975. Chromosome aberrations in somatic cells (in atomic bomb survivors). J. Radiat. Res. (Tokyo) (Suppl) 16:122-131.

Beebe, G. W. 1979. Reflections on the work of the Atomic Bomb Casualty Commission in Japan. Epidemiol. Rev. 1:184-210.

Beebe, G. W. 1981. Overall Risks of Cancer in A-Bomb Survivors and Patients Irradiated for Ankylosing Spondylitis. In H. Burchenal and H. F. Oettgen, eds. Cancer, Achievements, Challenges, and Prospects for the 1980's. Grune & Stratton, Inc., New York.

Beebe, G. W., and M. Usagawa. 1968. The Major ABCC Samples. Atomic Bomb Casualty Commission TR 12-68, Hiroshima.

Beebe, G. W., H. Kato, and C. E. Land. 1978. Life span study report 8: Mortality experience of atomic bomb survivors, 1950-74. Hiroshima. Radiat. Res. 75:138-201.

Cogan, D. G., S. F. Martin, and S. J. Kimura. 1949. Atomic bomb cataracts. Science 110:654-655.

Folley, D. G., W. Borges, and T. Yamawaki. 1952. Incidence of leukemia in survivors of the atomic bomb in Hiroshima and Nagasaki, Japan. Am. J. Med. 13:311-321.

Francis, T. Jr., S. Jablon, and F. E. Moore. 1959. Report of Ad Hoc Committee for Appraisal of ABCC Program, 1955. Atomic Bomb Casualty Commission TR 33-59, Hiroshima.

Kerr, G. D. 1979. Organ dose estimates for the Japanese atomic-bomb survivors. Health Physics 37:487-508.

Lifton, R. J. 1967. Death in Life: Survivors of Hiroshima. Simon and Schuster, New York.

Neel, J. V., and W. J. Schull. 1956. The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences, Washington, D.C.

Neel, J. V., H. Kato, and W. J. Schull. 1974. Mortality in children of atomic bomb survivors and controls. Genetics 76:311-326.

Neel, J. V., C. Satoh, H. B. Hamilton, M. Otake, K. Goriki, T. Kageoka, M. Fujita, S. Neriishi, and J. A. Asakawa. 1980. A search for mutations affecting protein structure in children of atomic-bomb survivors: A preliminary report. Proc. Natl. Acad. Sci. USA 77:4221-4225.

Oughterson, A. W., and S. Warren. 1956. Medical Effects of the Atomic Bomb in Japan. McGraw-Hill, New York.

Plummer, G. 1952. Anomalies occurring in children exposed in utero to the atomic bomb in Hiroshima. Pediatrics 10:687-693.

DISCUSSION

PROF. DARDANONI: Is there a common registration of patients in Japanese hospitals? If so, it would be rather easy (and inexpensive) to monitor morbidity.

DR. BEEBE: I know of no way to do this in Japan. There are insurance programs in Japan which, under other circumstances, might suit the need for morbidity data. As a substitute, we developed tumor registries and tissue registries, which are essentially tumor registries with histologic slides.

Reproductive Injury: Love Canal

David Axelrod[1]

The most likely source of human exposure to the chemicals deposited in the Love Canal landfill is through leaching of the compounds in soil, leading to high concentrations of chlorotoluene and chlorobenzene in the ambient air of nearby homes. Because fetuses are especially vulnerable to chemical insult, congenital defects, spontaneous abortions, and below-normal birth weight were selected as indicators of toxic human exposure. Adverse pregnancy outcomes were compared among matching groups in Canada, a neighborhood near but not contiguous to the landfill, and four sectors of the Love Canal area, two of them abutting the landfill. Findings suggest that an increased number of spontaneous abortions and/or low birth weights may have occurred in certain sections of the canal area. However, the results with respect to congenital defects were not statistically significant. The relationship of adverse pregnancy outcomes to evidence of chemical exposure has not been established.

The original geologic surveys of Love Canal are important to understanding the possible modes of human exposure and the ultimate effects of the chemicals on human reproduction. Soil strata at the canal site generally consisted of a thin mantle of silts and fine sands on top of low-permeability clay. The clay strata apparently acted as a barrier to water movement of chemicals below the surface. Rain and groundwater, accumulated either from natural or human activity, probably carried the chemical waste to layers of soil that had higher permeability, thereby facilitating the lateral migration of the pollutants.

---

[1] New York State Department of Health, Albany, N.Y.

The chemicals thus leached into the top soil layers and into the basements of nearby homes, resulting in human exposure to the contaminants. From the basements and top soil strata, the chemicals probably volatilized, producing high air concentrations of various agents, such as chlorinated organic compounds with low molecular weight.

Initial air samples, taken by the Environmental Protection Agency from the basements of 14 houses adjacent to the canal, showed the presence of 26 organic compounds. These samples showed clear evidence of chlorotoluene in the basement air of 33 houses (of 99 sampled) abutting the canal. Only four houses (of 256 sampled) in the outer areas of the landfill site showed trace evidence of contamination with chlorotoluene. There was similar evidence of the presence of chlorobenzene. Neither of these chemicals is commonly found in household products. Benzene, toluene, chloroform, and many other compounds were identified in a large number of homes, but the presence of these chemicals did not necessarily correlate with the presence of chlorotoluene or chlorobenzene.

Other migration mechanisms may also have been in effect. Chemicals may have traveled along surface paths, such as along the lowlands that were present before housing was built peripheral to the landfill. The topography of the region is generally flat, except for three creeks north of the canal. These creeks contain water throughout the year. Shallow depressions (swales) traversed the area; some transected the canal itself before the housing was built. The location of these swales was determined from aerial photographs, which were independently interpreted by personnel at Cornell University's School of Civil and Environmental Engineering. The university researchers had no prior knowledge of the swale hypothesis. The depressions could have served as drainage ways and even have produced ponds in some sections during times of high water. Additional verification of these historically "wet" areas was obtained by interviewing residents and reviewing their photographs and motion pictures.

Building construction has since modified the contour and extent of the swales. Housing built between 1951 and 1956 eliminated the major one, which had intersected the canal and carried surface water to peripheral areas. One 1966 aerial photograph shows only one small swale, northeast of the canal. At the time of this study, no visible, above-ground evidence remained of these natural swales.

Filling in the swales eliminated the potential chemical transport by actual surface flow, but not by migration. Permeable fill used in the swales may still have carried leachate from the canal. Furthermore, chemically contaminated soil may have been used to fill in low-level areas during development. For instance, major portions of the pond sections were filled during 1958. Minutes of a school board meeting in the 1950's record the transport of earth from the 99th Street schoolyard to the 93rd Street school property, beyond the area under study. Aerial photographs show that by May 1958, the vast majority of the historical depressions were leveled. A map of this area is shown in Figure 1 of my paper entitled "Chlorinated Hydrocarbons," which appears earlier in this volume.

Yet another migration mechanism was recently uncovered. Construction at 99th Street at the edge of the canal unearthed three pipes (10.0 cm in diameter) approximately 1 meter below the ground and extending laterally toward a former pond on the periphery of the study area. Farmers apparently had used these pipes to draw water from the canal before the chemical wastes were placed there.

Collectively, this information yields a relatively unambiguous picture of the original land surface, as modified by construction. And the hypothetical patterns of chemical migration along swales, through pipes, in earth transported elsewhere as fill could result in "preferential" contamination of the wet areas. Thus, for this study, housing was divided into two groups: all houses (116) built on wet (or water) areas (other than those adjacent to the canal itself), and those houses (268) built on historically "dry" (nonwater) areas. Soil samples were taken at every home to verify the former locations of ponds and swales and to determine the extent of chemical contamination of the soil. The samples were then divided into two groups -- undisturbed soil and disturbed soil, the latter samples containing at least some fill dirt. Houses with at least 1 meter of fill and houses adjacent to these were labeled "fill" houses. A high coefficient of agreement was found between fill houses and water houses. Comparison of a map of fill houses to a map of water houses substantiated the positive correlation, but other, more general modes of exposure and possible conduits of chemical migration were also considered. Residents of the area might have been exposed to toxic vapors emanating from the canal and transported by air or to contamination via the public water supply, in existence since 1930.

The study of the temporal pattern of housing development, the topography of the canal area, and the initial chemical evaluations suggested that many houses directly adjacent to the canal were contaminated. Pollution of the more peripheral areas would most likely have occurred through some general mechanism affecting broad areas or by a more selective route, such as the historic water area. Each street was examined separately in all statistical analyses because the temporal factors might be important and the houses on 99th Street were considerably older than those on 97th Street. Also, studying the area street by street might reveal a gradient effect as distances from the canal increased, which would support the hypothesis of some general mechanism of contamination. Historic water areas, where chemicals might have migrated from the canal or been brought in with contaminated fill, might show more evidence of contamination than the nonwater areas, which also generally had newer houses.

Specific biologic events--congenital defects, spontaneous abortions, and low birth-weight infants--were enumerated as possible end points of exposure, although the correlation of their incidence in humans to chemical exposure is not well established. However, these indicators have a shorter induction period than do most adult chronic diseases, and the prepartum period is generally accepted to be especially susceptible to chemical insult. It is agreed that many chemicals are hazardous to the conceptus of lower animals, depending on dosage, route of administration, and stage of gestation at exposure. Three end points were examined because of the wide variety of chemicals involved and their differing toxic manifestations. The monitoring of spontaneous abortion and the identification of environmental teratogens are important because the number and variety of congenital anomalies during gestation is far greater than can be detected from an analysis of full-term births.

Each of these end points has certain limitations. The frequency of spontaneous abortions, especially of those occurring very early in pregnancy, is exceedingly difficult to measure. Some congenital malformations, especially cardiac abnormalities and mental defects, are difficult to diagnose during the immediate postpartum period. Birth weight alone may not be a sufficiently sensitive or specific indicator of toxic effect.

Five epidemiologic hypotheses were advanced concerning possible distribution of spontaneous abortions, congenital defects, and low birth weights in specific sections of the study area. First, an increased incidence of some or all of the indicators might be demonstrated only among pregnant females residing in houses on 97th and 99th Streets, the two streets where backyards extend to the dump site and where the likelihood of chemical contamination was greatest. Second, an excess might also be observed in more peripheral areas. Examination of each indicator by location might point to a gradient effect as the distance from the canal increased. Third, the entire study area, including 97th and 99th Streets, might have an increased rate of all or some of the biologic indicators, suggesting a general mechanism of exposure, e.g., air, water. Fourth, increased incidence might be observed in the historic water areas of the neighborhood, and a lesser frequency in historic nonwater areas. Finally, the incidence rates might be related to temporal factors and to the nature and concentration of the chemicals to which pregnant females were exposed. For example, 99th Street might be characterized by an increased incidence of the most severe indicator, spontaneous abortion, and streets farther from the landfill might have an increased incidence of low birth weight infants.

Initially, expected numbers of spontaneous abortions were obtained from a report by Warburton and Fraser (1964). They studied a relatively large sample of the spontaneous abortion incidence in more than 6,000 Canadian pregnancies, tabulated both by birth order and maternal age at conception. However, possible differences between demography, economics, and other characteristics of this population and those of the Love Canal group dictated the need for additional control groups. Thus, a similar distribution table was constructed for residents of houses north of Colvin Avenue. This latter area, directly north of the canal, was selected because it has a large population residing in single-family houses near the canal. Most important, residents were concerned that chemicals might have migrated into their area. More than 98 percent of the adult residents from this area, as well as from the Love Canal site, participated in the investigation.

The validity of this comparison group is also limited. A greater proportion of the Colvin Avenue residents have college educations than do those in the entire study area, although this factor was controlled for in the statistical analysis. In addition, the area north of Colvin Avenue may also have been subject to chemical contamination. Were this so, it might lessen the extent to which the rate of abnormal reproductive events among Love Canal women appeared to deviate from the expected.

Finally, a maternal age parity table was constructed based on the spontaneous abortion experience of females residing in nonwater areas. This internal comparison group was used to evaluate further the hypothesis that an increased number of reproductive indicators might be present in historic water sections. But, again, the possibility of prior chemical contamination could not be excluded.

The same survey instrument was used throughout the investigation, with questions relating to medical, therapeutic, social, occupational, and pregnancy histories answered by all adult residents in a door-to-door survey. The same field teams were used in all areas studied. Investigators had no prior knowledge of the specific hypotheses under evaluation or of the areas selected as comparison groups. During the field work, supervisors, with at least 4 years of experience, reviewed completed questionnaires to assure standardized data collection. Two other supervisors and members of the statistical unit subsequently reviewed all questionnaires for completeness and possible inconsistencies.

A number of measures were followed to check validity of responses. The interview procedure had a built-in recall mechanism, and certain questions were asked repetitively in the questionnaire. Statistical analyses of the low birth weight indicator were performed separately on interview data and birth certificates obtained from the Office of Vital Records, and the results were compared. An effort was made to confirm all spontaneous abortions through vital records, physician interviews, and medical records. Questionnaires were keypunched in a key-to-disc unit with verification. Programs were checked by hand calculation to guarantee accurate implementation of computational algorithms. Statistical routines of sufficient complexity to defy hand computation were run against test data for verification.

Indicators were included only for those females who resided in the study area as of June 1978, and who lived there during the entire period of pregnancy. Spontaneous abortions were considered, confirmed, and included in analyses if they were verified by personal physicians or hospital records or if there was evidence of pregnancy with a subsequent history compatible with this outcome. Women who were ever pregnant at their present address in the study area were categorized according to their age at delivery and parity for each pregnancy.

For each of the areas studied, the number of miscarriages observed among the pregnancies was determined. The number of miscarriages expected among these women was calculated by applying the percentage of miscarriages for each parity combination of the other control groups to the observed number of pregnancies in each cell.

The expected numbers for all study areas were derived from the Warburton and Fraser report and from the controls in the Colvin area. The nonwater area was used as a control for the water area. The Mantel-Haensel chi square was used to test the differences between the observed and expected numbers of miscarriages.

Findings showed that, of the residents on the streets directly adjacent to the canal, only those on 99th Street may have had an excess of spontaneous abortions. The number of births in the water area was 80; in the nonwater area, 149. There was a much higher than expected incidence of untoward pregnancy outcomes in the wet households: 11 among 80.

With respect to low birth weights (defined as 2.49 kg or less), the relative risk of a small or a low birth weight infant from contaminated areas of the Love Canal was twice what was expected from a noncontaminated area. Comparisons were based on a 1977 New York State annual report. The New York State number was 3.16 kg for all white births; in the contaminated areas it was 6.31 kg, and in the noncontaminated areas it was 3.29 kg (New York State Department of Health, 1977).

The apparent increased rates of spontaneous abortions and low birth weights in the 99th Street and water areas may be due to some confounding variables or combination of factors such as maternal age and parity.

There was also the possibility that women who had one or more adverse pregnancy outcomes while residing in the Love Canal area might have had similar experiences before moving into the area. No significant number of prior spontaneous abortions was found using numbers from either the Warburton and Fraser report or those from the Colvin group. Prior low birth weights were not significantly increased when compared to rates from New York State. In addition, there was no documented evidence of congenital defects among the 57 live births to female residents of 99th Street and the water areas before they moved to the canal area. This finding is in juxtaposition to the 14 such episodes among 122 live births while these females resided in these two areas.

Workers in certain occupations, such as operating room attendants and chemical and laboratory operators, might also be subject to increased rates of spontaneous abortion and congenital malformation. Controlling for such exposure revealed no significant differences among households with either spontaneous abortions or congenital defects. There were too few households with a low birth weight child for the five-area chi square analysis.

Still other factors must be considered. Certain infections, such as rubella, mycoplasmas, and toxoplasmosis; prior, induced spontaneous abortions; alcoholism; and clinical conditions, including diabetes mellitus, epilepsy, malnutrition; and maternal exposure to lead, mercury, arsenic, or ionizing radiation, all might adversely influence pregnancy outcome. The medical histories of females having had spontaneous abortions and of children with either congenital defects or low birth weight were reviewed and, with one exception, none of these factors could be documented as pertinent.

Variability in interviewer technique and/or respondent's recall could also account for some of the differences. Analysis of the data by individual interviewer did not suggest that an increased incidence was attributable to any one interviewer. The time interval between the date of interview and the date of spontaneous abortion reported by women in the water and nonwater areas was examined. And, finally, a validity check suggested that the accuracy of respondent recall was comparable in the Love Canal and Colvin areas.

A comparison of the low birth weight information, as determined by interview data from each area, to the vital statistics data, revealed similar results. Only the water areas had a significant number of low birth weights when compared with the ratio of low birth weights recorded in New York State, excluding New York City.

Of all the factors, the most important were probably mother's age, parity, smoking, drinking, and social class, as indicated by educational status. House age and duration of residence were also important considerations.

In summary, these findings are consistent with the possibility that a slight to moderate increase in spontaneous abortions and/or low birth weight infants may have occurred on 99th Street and in historic water sections of the Love Canal area. Similar pregnancy outcome patterns have been observed among women living near a Swedish smelter that produces metallurgic and chemical products. Birth weight, obtained from vital records, is clearly the more objective and reliable indicator.

The results regarding congenital defects were not statistically significant, but the highest percentages were observed on 99th Street adjacent to the canal and in historic water sections.

Finally, and most important, geographic distributions of adverse pregnancy outcomes were not correlated with chemical evidence of exposure.

There is no direct evidence of a cause-effect relationship between chemicals from the canal and adverse pregnancy outcome.

REFERENCES

New York State Department of Health. 1977. Vital Statistics Report. Albany, N.Y.

Warburton, D., and F. C. Fraser. 1964. Spontaneous abortion risk in man: Data from reproductive histories collected in a medical genetics unit. Am. J. Human. Genet. 16:1-25.

DISCUSSION

DR. SUSKIND: What about possible chemical contamination from the swale area into the household? Very early in the study, the basements of some of the houses were examined for contaminants. Were basements of houses where families had reported birth defects examined for contaminants?

DR. AXELROD: We examined virtually all houses to determine the concentrations of chemicals. At this point, we cannot draw any conclusions about concentrations of various chemicals in the air with respect to the presence or absence of a congenital defect. Any air measurement is taken at one instant. Several times when we went back and repeated observations, samples produced different results.

DR. MOORE: Given the variety of chemicals that were found in Love Canal and given that your findings depended on numerous factors, do you ever expect to find a direct correlation between Love Canal exposures and a given birth defect or a low birth-weight child?

DR. AXELROD: It would be virtually impossible to exclude or include any individual case, but the accumulated epidemiologic evidence strongly suggests a correlation between an adverse response and exposure to the gamut of chemicals present in the area. We were unable to correlate a specific adverse response in our studies with respect to actual concentrations at a given location. A correlation is not excluded in the generic sense.

DR. MILLER: If you look over the list of situations that have been discussed involving PCB's, there was a transplacental effect.

With kepone, there was effect on fertility; with DBCP, an effect on sterility; with lead, an effect on the sperm and on the fetus; with methyl mercury, on the fetus. An effect on reproductive performance was seen -- except at Love Canal where there were 82 or more chemicals.

DR. AXELROD: There is, of course, a problem with small numbers in the study, and the exposures are very different in some respects from those at Yusho, Minamata, and the others. Those episodes involved relatively high dose levels. At Love Canal, we are considering a chronic lower level of exposure. In fact, the dose cannot even be measured. In the studies of atomic bomb effects, long latent periods have now elapsed, so effects can be found where information was previously lacking. There may or may not be a threshold at Love Canal. The sensitivity of the Love Canal studies to date is probably too weak to detect a low-dose effect on fertility rates.

DR. REGGIANI: Was TCDD among those chemicals found at Love Canal?

DR. AXELROD: Yes. We expected to find dioxin because of the kind of manufacturing process Hooker Chemical Company used at Niagara Falls. We also expected it when we learned of the large potential concentration of trichlorophenol. Subsequently, we found TCDD in several samples from areas adjacent to the canal and, also, on the bank of a stream north of the canal where there would have been sewer discharge.

Based on some grids we laid out, we believe that some construction work has prevented the TCDD from migrating as rapidly or as far as would normally be anticipated. For example, our samples turn from positive to negative at the end of the canal area, as if the chemicals did not cross the street. Construction of the street apparently served as a barrier, certainly to our analytic capability. The chemicals may be there but we cannot detect them. We could measure parts per trillion in the area adjacent to the road.

DR. REGGIANI: Was that the highest level that you measured?

DR. AXELROD: No, there were levels in the parts per billion range, as well. In the soil samples, as we moved toward the roadway, there were levels in the parts per trillion range. The difficulty is that we do not know exactly where the trichlorophenol was placed in the canal. If we could precisely isolate the area where the trichlorophenol was, we could probably get higher concentrations. Basically, we are sampling at random because the chemical distribution is unknown to us, and, I believe, unknown to anybody.

DR. REGGIANI: You expect a higher concentration?

DR. AXELROD: It is possible. Based on the levels of contamination known to be present in trichlorophenol, we would not be surprised to find considerably higher levels, at least in some of the chemical waste material that was removed from the canal proper. How much would have migrated, how much would have moved into the soil area, is anybody's guess.

DR. REGGIANI: I would expect concentrations at the parts per million level.

DR. AXELROD: I would not be surprised to find that. It depends on where you sample. We could be sampling in one area and find nothing, and we would sample 2 meters away and find significantly higher concentrations.

We don't know if disposal of chemicals in the canal was orderly at all times. According to some stories, the canal was partially filled in certain areas, with specific chemicals being placed in one area and not in another. We cannot confirm these.

DR. MOORE: Please elaborate on your concluding remark that there was no direct correlation or direct evidence of cause and effect.

DR. AXELROD: I did not mean it in the generic sense. In terms of soil sampling, and with respect to actual chemical concentrations in a given location, we were unable to correlate contamination with a specific adverse response. That is not to say, in the generic sense, that we do not believe that there is a correlation.

PROF. DARDANONI: What about fertility rates in the different groups?

DR. AXELROD: They are comparable, both with respect to the control groups and with respect to the various study populations we evaluated.

DR. MILLER: Would you recommend that studies be performed at other hazardous waste disposal areas?

DR. AXELROD: Something at the intensity of the study at Love Canal is probably not feasible on a grand scale. Where a reasonably direct population exposure can be assumed, then I think this sort of study has to be made. These sites have to be very carefully identified and selected because there simply are not enough resources to examine every one of the thousands of sites within the United States or even the hundreds of sites in New York State.

There is an absolute lack of data with which to compare the kind of information we accumulated at Love Canal. The first question to ask when you start comparing the outcomes of a study is what are you comparing them with? Certainly, the Warburton-Fraser report was not on an industrial community. The area north of Colvin was appropriate because it was in the same community. But there are legitimate questions that need to be raised regarding the kinds of outcomes for pregnancy in other industrialized areas where there is exposure to benzene, chlorotoluene, or any of a large variety of chemicals as a result of manufacturing processes or leaching.

Reproductive Injury: General Considerations

Robert W. Miller[1]

Reproductive injury may occur either before conception by affecting the germ cells, or after, by affecting the embryo or fetus. At the Atomic Bomb Casualty Commission in Japan, seven measures of germ-cell mutation have been studied: stillbirths and neonatal deaths, congenital malformations, birth weight, sex ratio, anthropometrics at about 9 months of age, F_1 mortality, and biochemical genetics. Sterility or infertility are other measures of either preconception or postconception effects. In addition, injury to the embryo may be measured by the frequency of miscarriages, malformations due to exposures while *in utero*, and transplacental carcinogeneiss. Observations in domestic or wild animals may provide clues to environmental agents that also cause harm to the human conceptus.

Reproductive injury occurs either before conception that is, by injury to germ cells, or after conception by injury to the embryo. The two possibilities are very different. The first involves a germ-cell mutation; the second, an embryologic catastrophe affecting the organism *in utero*.

STERILITY, INFERTILITY, AND MEASURES OF GERMINAL MUTATIONS

One measure of reproductive injury is sterility: no sperm yields no baby. Another possible effect is infertility: a diminished number of sperm diminishes the chance of having children.

The most comprehensive measurements of human genetic damage have been made by the Atomic Bomb Casualty Commission (now called the Radiation

---

[1] Clinical Epidemiology Branch, National Cancer Institute, Bethesda, Md.

Effects Research Foundation) in Japan. The study by Neel and Schull (1956) involved six indicators of genetic damage observed in 70,000 children conceived after the bombs were dropped. Only a small percentage of the parents had been heavily exposed.

No excess in malformations was observed, nor was there an increased incidence in the frequency of stillbirths and neonatal deaths combined. However, any increase less than 1.8-fold would have gone undetected. There were also no effects attributable to radiation in the third and fourth measures of genetic effects--birth weight and measurements at 8 to 10 months of age. The fifth--disturbance in the sex ratio, depending on whether the mother or father was exposed--was observed in the early years; but this finding, of uncertain significance, disappeared 10 years later. The sixth--death rate in the generation of children conceived after exposure--also showed no effect.

Recently, a seventh measure involving biochemical genetics has been developed (Neel *et al.*, 1980). An electrophoretic study is made of proteins in the child's blood. An abnormality found in red cells or plasma that is not present in the parents' blood is presumed to be a mutation attributable to the exposure.

EMBRYOTOXICITY AND TERATOGENESIS

Measuring the effects of postzygotic exposures includes recording the frequency of miscarriages, which are very difficult to evaluate because women often do not know they are miscarrying, especially during early pregnancy. Also, a woman may provide very different responses at different times. Hospital records, of course, do not routinely provide information about miscarriages that occur at home.

Environmental effects on the embryo during the first weeks of pregnancy may be lethal (embryotoxic). Later in the first trimester, during the formation of body organs, such effects can cause congenital anomalies (teratogenesis). Small head circumference and mental retardation following intrauterine radiation exposure were known before the atomic bomb exposures in Japan. Fourteen case reports related to pelvic radiotherapy had been collected by 1928 (Murphy, 1929). Subsequently, an inexpensive mail survey of obstetric services in the United States located 16 more children with small head circumference and mental retardation whose mothers had received pelvic radiotherapy (Goldstein and Murphy, 1929).

Thus, pediatricians with the Atomic Bomb Casualty Commission knew to look for these effects, and found them in children exposured to radiation before they had reached 18 weeks of gestational age (reviewed by Miller and Mulvihill, 1976). The effect was related to dose.

In Hiroshima, exposures before the fourth week of gestational age apparently had a lethal effect. Exposure between the 4th and the 17th weeks induced small head circumference in a substantial proportion of infants; the frequency and severity of this effect increased as the dosage increased. The effect in Hiroshima was detectable even among children whose mothers had received only 10-19 rads in air; the embryo had received less because the mothers' bodies had attenuated the dose. Higher doses, beginning at about 50 rads, were associated with mental retardation. In Nagasaki, fewer pregnant women were exposed; small head size and mental retardation of their infants were not associated with a dosage of less than 150 rads (Blot and Miller, 1973; Miller and Blot, 1972).

Polychlorinated biphenyls (PCB's) cross the placenta and can produce a teratogenic effect. Affected infants are small for date, have transient hyperpigmentation, and eyes swollen by Meibomian cysts. In two of nine cases reported by Taki et al. (1969), teeth were present at birth.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is teratogenic in laboratory animals and was reported by Kimbrough et al. (1977) to cause unspecified malformations in horses. In Moscow Mills, Mo., a waste-oil dealer mixed TCDD with oil and disposed of it illegally by spreading it on the earth in horse arenas. As a consequence, 43 horses died and 26 others aborted. Many foals were stillborn or died soon after birth (Kimbrough, 1977).

TRANSPLACENTAL CARCINOGENESIS

Chemicals that cross the placenta can induce cancer in offspring. The first transplacental carcinogen recognized in humans was diethylstilbestrol (DES). In Boston, seven cases of a rare tumor (adenocarcinoma of the vagina) were observed in young women within a 4-year period. Retrospective study identified DES, given therapeutically to their mothers during pregnancy, as the cause (Herbst et al., 1971). In fact, a mothers whose daughter was affected suggested DES to the medical investigators who were on the watch for such an explanation (Ulfelder, 1980). The relationship was quickly confirmed by the New York State Tumor Registry (Greenwald et al., 1971). The risk eventually was shown to be about 1 per 1,000 DES daughters (Herbst et al., 1977).

After the transplacental carcinogenic effect of DES was discovered, a high proportion of the women who had been exposed in utero were found to have congenital anomalies of the lower genital tract (Herbst et al., 1975). An anomalous contour of the uterine cavity, recorded on hysterosalpingograms, has now been observed and is associated with abnormal pregnancy outcomes (Kaufman et al., 1977). A recent followup of daughters whose mothers participated in a clinical trial of DES given randomly during pregnancy several decades ago revealed that the unfavorable pregnancy outcome was related to DES (Herbst, 1980). These results from a clinical trial are not in accord with the suggestion by Barnes et al. (1980) that the effect on outcome of pregnancy may be heritable.

Among other possible transplacental carcinogens is diphenylhydantoin (DPH), prescribed for epilepsy. Taken during pregnancy, DPH occasionally produces a syndrome of birth defects, including hypoplasia of the midface and hypoplasia or aplasia of the fingernails and toenails (Hanson and Smith, 1975). Occasionally, it induces lymphoma in adults (Hoover and Fraumeni, 1975). This observation led to the expectation that lymphoma might occur at an increased rate in children with the fetal hydantoin syndrome. Four children have now been reported with the syndrome, but neuroblastoma, not lymphoma, was observed (Allen et al., 1980). In the fourth case, three older siblings were unaffected although the mother received DPH throughout all pregnancies. There may be an intrauterine interaction involving other environmental factors or genetic susceptibility of the fourth child. Letters to Lancet detailed the first two cases. Repeated appeals for more information about other cases led to the report of the third. Publication of this case drew a report of a fourth case (summarized by Allen et al., 1980). Thus, what is probably the second known transplacental carcinogen in humans was identified by an approach that one might call "microepidemiology."

VETERINARY OBSERVATIONS

An article published in Modern Veterinary Practice (Crowe, 1969) described five epidemics of congenital arthrogryposis in pigs on Kentucky farms, perhaps due to exposures to pesticides or growth stimulants used on tobacco (Crowe and Swerezek, 1974). Similar episodes occurred in Missouri, where ingestion of wild black cherries was implicated (Selby et al., 1971). No comparable human effects are known, but such observations in domestic (or wild) animals may serve as indicators of potential embryologic hazards in humans.

Chemicals can cross the placenta and produce death, deformity, or cancer in the embryo or fetus. They can also be transmitted by lactation, through the fat of breastmilk in which PCB's, TCDD, polybrominated biphenyls (PBB's) and pesticides are concentrated. Finally, in theory at least, the embryo or fetus can be affected by dust brought home on a parent's workclothes. No examples of embryologic effects are known, but asbestos has been transmitted to children this way in several households, and decades later those persons developed mesothelioma (Anderson et al., 1976; Li et al., 1978). Chisolm (1978) has referred to such contamination of the home as fouling one's own nest.

REFERENCES

Allen, R.W., Jr., B. Ogden, F.L. Bentley, and A.L. Jung. 1980. Fetal hydantoin syndrome, neuroblastoma, and hemorrhagic disease in a neonate. J. Am. Med. Assoc. 244:1464-1465.

Anderson, H.A., R. Lilis, S.M. Daum, A.S. Fischbein, and I.J. Selikoff. 1976. Household-contact asbestos: Neoplastic risk. Ann. N.Y. Acad. Sci. 271:311-323.

Barnes, A.B., T. Colton, J. Gundersen, K.L. Noller, B.C. Tilley, T. Strama, D.E. Townsend, P. Hatab, and P.C. O'Brien. 1980. Fertility and outcome of pregnancy in women exposed in utero to diethylstilbestrol. N. Engl. J. Med. 302:609-613.

Blot, W.J., and R.W. Miller. 1973. Mental retardation following in utero exposure to the atomic bombs of Hiroshima and Nagasaki. Radiology 106:617-619.

Chisolm, J.J., Jr. 1978. Fouling one's own nest. Pediatrics 62:614-617.

Crowe, M.W. 1969. Skeletal anomalies in pigs associated with tobacco. Mod. Vet. Pract. 50:54-55.

Crowe, M.W., and T.W. Swerczek. 1974. Congenital arthrogryposis in offspring of sows fed tobacco (Nicotiana tabacum). Am. J. Vet. Res. 35:1071-1073.

Goldstein, L., and D.P. Murphy. 1929. Etiology of the ill health in children born after maternal pelvic irradiation. Part II: Defective children born after post-conception pelvic irradiation. Am. J. Roentgenol. 22:322-331.

Greenwald, P., J.J. Barlow, P.C. Nasca, and W.S. Burnett. 1971. Vaginal cancer after maternal treatment with synthetic estrogens. N. Engl. J. Med. 285:390-392.

Hanson, J.W., and D.W. Smith. 1975. The fetal hydantoin syndrome. J. Pediatr. 87:285-290.

Herbst, A. L. 1980. A comparison of pregnancy experience in DES-exposed and DES-unexposed daughters. J. Reprod. Med. 24:62-69.

Herbst, A.L., H. Ulfelder, and D.C. Poskanzer. 1971. Adenocarcinoma of the vagina. N. Engl. J. Med. 284:878-881.

Herbst, A.L., D.C. Poskanzer, S.J. Robboy, L. Friedlander, and R.E. Scully. 1975. Prenatal exposure to stilbestrol: A prospective comparison of exposed female offspring with unexposed controls. N. Engl. J. Med. 292:334-339.

Herbst, A.L., P. Cole, T. Colton, S.J. Robboy, and R.E. Scully. 1977. Age-incidence and risk of diethylstilbestrol-related clear cell adenocarcinoma of the vagina and cervix. Am. J. Obstet. Gynecol. 128:43-50.

Hoover, R., and J.F. Fraumeni, Jr. 1975. Drugs. Pp. 185-198 in J.F. Fraumeni, Jr., ed. Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control. Academic Press, Inc., New York.

Kaufman, R.H., G.L. Binder, P.M. Gray, Jr., and E. Adam. 1977. Upper genital tract changes associated with exposure in utero to diethylstilbestrol. Am. J. Obstet. Gynecol. 128:51-59.

Kimbrough, R.D., C.D. Carter, J.A. Liddle, R.E. Cline, and P.E. Phillips. 1977. Epidemiology and pathology of tetrachlorodibenzodioxin poisoning episode. Arch. Environ. Health 32:77-86.

Li, F.P., J. Lokich, J. Lapey, W.B. Neptune, and E.W. Wilkins, Jr. 1978. Familial mesothelioma after intense asbestos exposure at home. J. Am. Med. Assoc. 240:467.

Miller, R.W., and W.J. Blot. 1972. Small head size after in-utero exposure to atomic radiation. Lancet 2:784-787.

Miller, R.W., and J.J. Mulvihill. 1976. Small head size after atomic irradiation. Teratology 14:355-357.

Murphy, D.P. 1928. Ovarian irradiation, its effects on the health of subsequent children: Review of the literature, experimental and clinical, with a report of 320 human pregnancies. Surg. Gynecol. Obstet. 47:201-215.

Neel, J.V., and W.J. Schull. 1956. The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences, Washington, D.C. 241 pp.

Neel, J.V., C. Satoh, H.B. Hamilton, M. Otake, K. Goriki, T. Kageoka, M. Fujita, S. Neriishi, and J. Asakawa. 1980. Search for mutations affecting protein structure in children of atomic bomb survivors: Preliminary report. Proc. Natl. Acad. Sci. USA 77:4221-4225.

Selby, L.A., R.W. Menges, E.C. Houser, R.E. Flatt, and A.A. Case. 1971. Outbreak of swine malformations associated with the wild black cherry, Prunus serotina. Arch. Environ. Health 22:198-201.

Taki, I., S. Hisanaga, and Y. Amagase. 1969. Report of Yusho (chlorobiphenyls poisoning): Pregnant women and their fetuses. Fukuoka Igaku Zasshi 60:471-474.

Ulfelder, H. 1980. The stilbestrol disorders in historical perspective. Cancer 45:3008-3011.

DISCUSSION

DR. REHDER: With respect to leukemia after radiation of newborn children, do you think there are two different pathomechanisms of inducing tumors by exposing fetuses to certain substances? In some cases, is there a direct oncogenic effect as, perhaps, in the leukemias? In other cases, is there dysgenesis that increases the risk of tumor?

DR. MILLER: Yes, leukemia may have, as its basis, a chromosomal abnormality induced by an agent, such as radiation, and then a clonal evolution of the leukemic cell takes place. The other mechanism (dysgenesis) seems clear from studies of human embryos. Areas of malformed tissue, for example, have been seen under the capsule of the kidney. In patients with Wilms' tumor of both kidneys (multifocal Wilms' tumor), dysplasia regularly found under the capsule of the kidney is believed to be due to a germinal mutation, and a second postgygotic event causes malignant transformation. But that second event does not have to happen.

Yes, there appear to be different mechanisms. Immunosuppression may be one; another may relate to enzymes in the embryo or the fetus. As I understand it, enzymes develop late in fetal life. If enzyme activation is required for carcinogenesis, exposure before then might not have an effect.

Cancers induced by environmental exposure of fetuses may not appear for decades, as has happened in experimental studies with nitrosamines, for example. In DES-induced cancer, the cancers appear mostly in women from 14 to 29 years of age. So, one may have to wait a long time to see what, if any, effect there is.

Reproductive Injury: General Considerations

Helga Rehder[1]

Reproductive injuries are frequent events. Approximately 20% of all conceptions are spontaneously aborted, and 30% of all stillborn and 2% of all liveborn infants display major congenital malformations. It is important to delineate disorders caused by exogenous agents from those that are genetically determined or due to recognizable chromosomal aberrations. Proving the association of exogenous disturbances with known environmental contamination is difficult. Experimental background information on the biologic effects of a compound is helpful, but the results may not be absolutely valid when related to other species. Mutations may not appear when exposed cells are susceptible to lethal cytotoxic side effects. A teratogenic potency, which is claimed to be nonspecific for any given substance, depending only on the sensitive periods of organ development at the time of exposure, may seem to gain specificity because fetal uptake of different substances occurs at different developmental periods and there may be selective accumulation in a single fetal tissue. The possibility of indirect fetal damage also has to be considered for those compounds that cause lesions of the yolk sac endoderm or of the placenta, rather than direct injury to the embryo. Time and grade of exposure as well as the type and pattern of developmental disturbance must be analyzed for each case in order to evaluate environmental reproductive hazard.

The modes of detecting reproductive injuries caused by environmental contamination are diverse. Attention may first be attracted by a sudden and striking increase in the number of certain congenital anomalies such as occurred near Minamata Bay in Japan from 1953 to 1960. Methylmercury was later identified as the pollutant causing the birth defects (Matsumoto *et al.*, 1965; Tatetsu and Harada, 1968). A similar pattern of malformations led to the discovery of the reproductive effects of thalidomide (Lenz and Knapp, 1962). In both of these instances, reproductive injuries occurred, and the causes were traced.

---

[1] Institute für Pathologie der Medizinischen Hochschule Lübeck, Lübeck, Federal Republic of Germany.

More difficult is the search for after-effects of a known accident, such as the tetrachlorodibenzodioxin contamination at Seveso (Hay, 1976, 1977a,b; Rehder et al., 1978), or a correlation of an increased number of animal deaths in areas of concentrated lead industry to some effect on humans (Koch and Vahrenholt, 1978).

In the first type of study, a pattern of congenital defects seems to follow a homogeneous pattern, and the effect of a pollutant on reproduction seems obvious. For the second type, effects seem heterogeneous, and any congenital defect is automatically ascribed specifically to the polluting event. Assessment of abnormalities is based mainly on epidemiologic data such as fertility pattern, spontaneous abortion rate, frequency of malformations, neonatal and childhood mortality, and even childhood malignancies within the exposed group. Exact data, however, may be unavailable; after a polluting event, the exposed population and physicians tend to overreport reproductive failures. In normal times, underreporting is more common. According to a study in Pennsylvania reported by Babbot and Ingalls (1962), only 60% of malformed liveborn babies were reported as malformed on their birth certificates. Furthermore, exact control data are often missing. Centralized monitoring of birth defects has only just started in a few countries (Ericson et al., 1977; Klingberg and Papier, 1979).

In addition, too little consideration is given to the fact that prenatal survival is itself the outcome of natural selection. Developmental failures are frequent events, occurring spontaneously in any population and area and without recognizable environmental hazards.

In humans, at least 20% of all conceptions are spontaneously aborted (Tuchmann-Duplessis, 1975) and, although only few systematic embryopathological investigations have been performed, it is apparent that the number and variety of anomalies during gestation is far greater than can be recognized from an analysis of term births (Stein *et al*., 1975). Even animals have a constant spontaneous abortion rate. In the mouse, it is 5% to 8%, the variation dependent on whether the animal is born in the wild or in the laboratory (Berry and Peters, 1976).

Anomalies are probably caused by a number of factors such as hormonal changes, intrafollicular aging of the ovum, delayed fertilization, unfavorable uterine environments, infections, other maternal disorders, immunologic incompatibilities, spontaneous genetic mutations, and--last but not least--chromosomal abnormalities. Extensive cytogenetic studies of almost 1,500 clinical cases of spontaneous abortions between the 3rd and 12th week showed chromosomal abnormalities in 60% of the specimens younger than 7 weeks. Whereas, in abortions between the 8th and 12th gestational week, the frequency was 23% (Boue and Boue, 1976). My own experiences indicate that the incidence of chromosomal aberrations is also very high in abortions occurring in late pregnancy. Among newborns, the rate is only 0.5% (Hamerton *et al*., 1975).

Although selective elimination of conceptus with congenital malformations proceeds during prenatal life, there still remain many deformed fetuses that survive until birth. Major congenital malformations are observed in 30% of all stillborn and in 2% of all liveborn infants.

Minor anomalies are found in 10% to 15% of newborns (Marden et al., 1964). These numbers may vary among different obstetric and neonatal units and among different areas, as was demonstrated most impressively by the 40-fold difference found in the frequency of anencephaly between the highest in Belfast and the lowest in Bogota and Ljubljana (Stevenson et al., 1966).

To evaluate environmental reproductive hazards, it is important to differentiate the cases with reproductive injury caused only by exogenous influences from those caused by endogenous disorders. This requires the ability and experience to recognize, for example, genetic disorders in the fetus. However, since a chemical agent may become effective via endogenous disturbances, experimental background information on a compound's biologic effects is necessary.

A chemical agent may act as mutagen, teratogen, and fetotoxin. A mutagen influences reproduction by affecting germ cells; it may express itself as a point or gene mutation, as a chromosome mutation, and as a genome mutation. Single-point or gene mutations generally have no effect on the individual and are seen as normal polymorphisms. They will not be recognized as long as they represent recessive gene mutants. When these genes accumulate within a population, they may eventually appear in the homozygous state in later generations. Some of the dominant gene mutants, however, may result in congenital diseases of fetuses. These are known as genetic diseases and are recognizable as such in many cases. More than 1,200 autosomal dominant and 900 autosomal recessive, and approximately 170 X-linked genetic disorders are recognized (McKusick, 1975). When point mutations have accumulated within a fertilizing germ cell, they exhibit a lethal effect on the heterozygous zygote. It has to be mentioned in this context that the "spontaneous mutation rate" is very high, varying between 2 to 3 per million gametes for hemophilia B and 100 per million gametes for neurofibromatosis (Vogel, 1970).

Chromosome mutations affect the actual structure in the form of deletions, duplications, inversions, and translocations following chromosome breaks. When genetically imbalanced, they may kill the gamete or the early embryo. Cytogenetic studies show chromosome mutations in 10-15% of second trimester abortions (Ruzicska _et_ _al_., 1970); among newborns, only 2% carry a structural, mainly balanced aberration (Hamerton _et_ _al_., 1975).

Genome mutations change the chromosome number. They result from extrachromosomal disturbances, either from meiotic nondisjunction when single chromosomes are lost or triplicated or from interference with cell cleavage in cases of polyploidy. Most genome mutations are lethal. Very few affected fetuses (such as those exhibiting trisomy 21, 18, or 13) survive until birth.

There are a number of _in_ _vivo_ and _in_ _vitro_ test systems for mutagenicity using microorganisms or somatic or germ cells of plants, insects, and mammals. The most relevant one for humans is the dominant lethal test. Thus, it was shown that metals such as mercury, lead, and cadmium do not induce point or chromosome mutations in the mammalian germ cell test (Gebhart, 1977). These metals interfere with chromosomal segregation during mitosis in human leukocytes (Fiskesjo, 1970) and in _Drosophila_ (Ramel _et_ _al_., 1969). However, these results cannot very well be extrapolated to meiosis since none of these substances has, up to now, shown a lethal effect in the dominant lethal test--an effect that would be expected from the genome mutation in germ cells.

Chlorinated carbohydrates may be another example of compounds that do not show mutagenic effects in the dominant lethal test system. Nonetheless, wives whose husbands were exposed to vinyl chloride monomers at their workplace have an increased abortion rate (Infante *et al.*, 1976). Abortion rates in wives of unexposed workers at the same plant were within normal limits, so the increase in the fetal wastage is attributed to mutation of male germ cells.

Although one may assume that many chemical agents have a mutagenic effect on human reproduction and that at least some of the so-called spontaneous mutations are actually exogenously induced, possibly by chemical compounds. There has been no evidence of an increase of genetic or chromosomal diseases in areas of detectable chemical contamination. Such findings may be obscured by a high dominant lethal effect or to a high spontaneous abortion rate. Unfortunately, there have been no thorough investigations of abortion material for confirmation.

Furthermore, it has to be considered that mutagenic effects may not be expressed when exposed cells undergo regression. This finding became evident among pesticide workers who developed oligospermia or aspermia when they were exposed to dibromochloropropane (DBCP) (Biava *et al.*, 1978; Whorton and Milby, 1980). Investigators used x-rays and gamma rays to show that spermatogonia, representing reproductive stem cells, as well as dictyotene oocytes, are far more susceptible to cytotoxic rather than to mutagenic effects. They also showed that it is easier to induce mutations in more differentiated germ cells (such as spermatocytes, spermatids, and in fertilized ova during the pronucleus phase, that is, before fusion of the two nuclei) (Vogel *et al.*, 1969). The conclusion is that, if there is a mutagenic effect on germ cells, it is temporary, resulting mainly in a reduction of fertility.

Mutagenic effects on the embryo itself (on embryonic somatic cells) should result in genetic mosaicism, relevant only when occurring in very early embryonal stages. Again, it was shown that in these, the cytotoxic effect predominates (Brent, 1979; Russel, 1965) and that preimplantation embryos respond to cytotoxicity with an "all or nothing effect," either being killed or progressing into an apparently normal individual. Possible tumor induction by mutation of somatic cells in older fetuses will not be discussed in this paper. The lack of more differentiated sensible germ cell stages in the fetus limits the possible mutagenic effect on fetal germ cells.

Teratogenic effects of a chemical compound on human reproduction are relatively easy to detect. "Teratogenesis" refers to impairment of embryonal organogenesis within a certain early developmental period; impairment of growth and differentiation or maturation in later developmental periods is a fetotoxic effect. This distinction is important with respect to the higher sensitivity and regenerative potency of the undifferentiated embryonal cell and thus to the type of the resulting anomaly.

Teratogenic compounds, if exerting direct action on the embryonal tissue, interfere with embryonal metabolism. They may cause nonspecific alterations, such as retarded development of the primordia by arresting or retarding mitotic activity, by killing cells, by inhibiting cell migration or cell interaction, or by disturbing inductional processes (Nishimura and Tanimura, 1976). Resulting organ anomalies are generally present as "defect malformations", but they also appear as "augmentation malformations" through overcompensation of early regenerative processes (Merker, 1977). The type of developmental defect does not reflect the exact temporal onset of the fetal injury, which generally occurs earlier than the developmental process, which is disturbed. This delayed effect is due to the attempted regenerative proliferation of the surrounding primordial cells that precedes the development breakdown and that may even fully compensate for the defect.

Despite their different biochemical pathways, different chemical agents can cause the same types of malformations if exposure occurs with the same sensitive period of organogenesis. However, some types of defects occur more frequently than others. Neural tube defects or cleft palate can be induced experimentally in almost any animal species by many different substances. These defects are the first to attract attention in areas of suspected chemical contamination such as occurred in Ohio in 1975, where the occurrence of neural tube defects was attributed to the presence of the polyvinylchloride industry (Edmonds _et al._, 1975). In New Zealand and Sweden, the same defects were ascribed to the spraying of forests with 2,4,5-trichlorophenol (2,4,5-T) (Division of Public Health, New Zealand, 1977; Swedish National Board of

Health and Welfare, 1977). A rise in the incidence of cleft palate was also correlated to the use of 2,4,5-T (Nelson et al., 1979). Since palatine shelf and neural tube closure obviously represent extremely sensitive developmental processes, neural tube defects and cleft palate may also result from unspecific disturbances. This hypothesis is supported by the high incidence of these malformations in discordant homozygous twins (Bellefeuille, 1969) and by their association with many chromosomal syndromes, where the malformations are not directly genetically determined but result from a general retardation of cell proliferation.

A chemical agent may gain some teratogenic specificity by strongly and selectively accumulating in single fetal tissues. Thus, experimentally verified localization of tetracycline in fetal bone structures (Andre, 1956; Blomquist and Hangren, 1966), of thiouracil in the thyroid (Slanina et al., 1973), and of some polycyclic compounds (chloroquine and chlorpromazine) in the melanin of the eye and in the inner ear (Dencker and Lindquist, 1975; Denecker et al., 1975; Lindquist and Ullberg, 1972) can be correlated to reported organ damage in human fetuses after application of these substances to pregnant mothers. However, the main sites of accumulation are the placenta and the yolk sac. Fetal uptake of the different compounds occurs from these sources at different developmental stages, thus limiting and specifying their teratogenic effect.

An earlier placental barrier and an accelerated fetal uptake with advancing gestation has been shown in mouse and hamster for 2,4,5-T (Dencker, 1976). This finding implies an effect on the fetus only in the late organogenic phase and explains the experimental induction of cleft palate or hydronephrosis. Neural tube defects would not, however, fall into that category because they represent disturbances during early organogenesis.

In contrast to 2,4,5-T, cadmium acts only in the early organogenic period. It does not cross the placenta at all. Apparently, it enters the fetal gut via the yolk sac just before closure of the vitelline duct. Its teratogenic effect then decreases rapidly (Denecker, 1975). The experimental finding of cleft palate as a late organogenic disturbance resulting from cadmium application is explained by the intimate contact of the stomodeum and foregut before the formation of the oral and pharyngeal cavities.

Salicylic acid has free passage to the fetus throughout gestation, and it is taken up to a similar degree at all stages. Thus, it may cause a wide range of malformations and fetotoxic effects. The structure most severely damaged will be the one undergoing most rapid development at the time of administration (Kimmel et al., 1971). Benzoic acid, widely used as a food preservative, increases the embryonic concentration of salicylic acid.

Embryonic uptake of inorganic mercury varies in relation to placental development. In early gestation, the substance may enter the fetal gut via the yolk sac (like cadmium), but it exhibits less teratogenic activity. Closure of the vitelline duct temporarily arrests the passage of mercury, but increasing fetal concentrations are again observed in late gestation (Dencker, 1976). The more lipid-soluble organic mercury compounds, such as methylmercury, are much more readily transferred to the fetus and are teratogenic during the entire organogenic period.

A chemical compound can also act on organogenesis if the primary target is not the embryonic cell but, rather, certain maternal tissues or the placenta. Inhibited embryotrophic nutrition after chemical compounds have accumulated within the placenta may, secondarily, lead to fetal growth retardation or even to fetal malformations. This pathomechanism has been proposed for the action of trypan blue on the fetus (Beck _et_ _al_., 1967). Furthermore, researchers have shown that, after application of methylmercury, a distinct decrease of fetal amino acids can be related, at least in part, to impaired placental transfer (Olson and Massaro, 1978).

Although the teratogenic effects of a compound are confined to the period of organogenesis (leading to a reduction of still undifferentiated primordial cells and, thus, to a true malformation), _fetotoxic damage_ in later gestation is characterized by a disturbance in body and organ growth and by destruction of more or less differentiated tissues. These alterations are followed by hypotrophy and by a change in the distribution of parenchymal versus connective (mesenchymal or glial) tissue, which results in a reduction of organ function and--in more extended tissue defects--in either secondary cyst formation or scarring. The fetal brain seems to be the most susceptible to toxic damage and may display a wide range of congenital alterations. Microencephaly and microgyria, due to a narrowing of the cerebral and cerebellar cortex and to nerve cell necrosis, have been described in congenital Minamata disease (Matsumoto _et al._, 1965). Experimental lead intoxication of pregnant rats and chick embryos resulted in brain hemorrhage and in hydrocephaly due to obstruction of the aqueduct (Ridgway and Karnofsky, 1952). In some instances, cystic porencephaly, following more extended destructive brain lesions in midterm fetuses (when the reactivity of the glial tissue is still limited), may be related to exogenous disturbances. Hydranencephaly or even ulegyria, occurring in older fetuses when reactive gliosis responds to the tissue damage, may also be related to exogenous disturbances. Extracerebral fetotoxic lesions can be observed in the lens of the eye (Brent, 1979), in the lymphoid and hematopoietic tissue (Driscoll _et al._, 1963), and--mainly by the way of circulatory disturbances--in the liver, heart, and lungs (own observations), where parenchymal defects are replaced by mesenchymal proliferations leading to tissue fibrosis and hamartomatosis.

Again, one has to consider placental accumulation of a chemical compound to be responsible, at least partly, for the fetotoxic effects such as hypotrophy and fetal death, even in those cases in which the fetotoxic agent can be traced in placental as well as in fetal tissues.

This rough diagram of possible pathomechamisms of mutagenic, teratogenic, and fetotoxic compounds is not complete. Rather, it is meant to focus on difficulties of their recognition and to emphasize the many different aspects that must be considered before reproductive injuries can be related to a specific environmental contamination. Epidemiologic data and experimental background information on the biologic effects of chemical compounds is helpful, but are often insufficient and not of absolute validity when extrapolated to humans or to conditions outside the laboratory.

In fact, there has been little evidence of reproductive injury resulting from chemical contaminants mainly because of the lack of systematic cytogenetic, toxicologic, and pathoanatomical investigations of fetal material in humans. It should be emphasized that morphologic and cytogenetic analyses, even of early fetuses, are possible and are important. Only when pathomechanisms and effects of certain compounds on human reproduction have been analyzed can one develop preventive efforts, which may even include the use of selective antimutagenic and antiteratogenic substances.

This work was supported by the Deutsche Forschungsgemeinschaft Re 429/3.

REFERENCES

Andre, T. 1956. Studies on the distribution of tritium-labelled dihydrostreptomycin and tetracycline in the body. Acta Radiol. (Diagn.) Suppl. 142:1-89.

Babbot, J.G., and T.H. Ingalls. 1962. Field studies of selected congenital malformations occurring in Pennsylvania. Am. J. Public Health 52:2009-2017.

Beck, F., J.B. Lloyd, and A. Griffiths. 1967. Lysosomal enzyme inhibition by trypan blue: A theory of teratogenesis. Science 157:1180-1182.

Berry, R.J., and J. Peters. 1976. Genes, survival and adjustment in an island population of the housemouse. Pp. 23-48 in S. Karlin and E. Nevo, eds. Population Genetics and Ecology. Academic Press, New York.

Biava, C.G., E.A. Smuckler, and D. Whorton. 1978. The testicular morphology of individuals exposed to dibromochloropropane. Exp. Mol. Pathol. 29:448-458.

Blomquist, L., and A. Hanngren. 1966. Flourescence technique applied to whole body section for distribution studies of tetracyclines. Pharmacology 15:215-219.

Boue, J.G., and A. Boue. 1976. Chromosomal anomalies in early spontaneous abortion. Pp. 193-208 in A. Gropp and K. Benirschke, eds. Current Topics in Pathology. Vol. 62, Springer, New York.

Brent, R.L. 1979. Effects of ionizing radiation on growth and development. Contrib. Epidem. Biostatist. 1:147-183.

deBellefeuille, P. 1969. Contribution à l'étiologie de l'anencéphalie par l'étude des gumeaux. Union Med. Can. 98:437-443.

Dencker, L. 1975. Possible mechanisms of cadmium fetotoxicity in golden hamsters and mice: Uptake by the embryo, placenta and ovary. J. Reprod. Fertil. 44:461-471.

Dencker, L. 1976. Tissue localization of some teratogens at early and late gestation related to fetal effects. Acta Pharmacol. Toxicol. 39 (Suppl. I):5-131.

Dencker, L., and N.G. Lindquist. 1975. Distribution of labelled chloroquine in the inner ear. Arch. Otolaryngol. 101:185-188.

Dencker, L., N.G. Lindquist, and S. Ullberg. 1975. Distribution of an ^{125}I-labelled chloroquine analogue in a pregnant Macaca monkey. Toxicology 5:255-264.

Division of Public Health, Department of Health, New Zealand. June 1977. 2,4,5-T and human birth defects: A report. Division of Public Health, New Zealand. pp. 2.

Driscoll, S.G., S.P. Hicks, E.H. Copenhaver, and C.L. Easterday. 1963. Acute radiation injury in two human fetuses. Arch. Path. (Chicago) 76:113-119.

Edmonds, L.D., H. Falk, and J.E. Nissim. 1975. Congenital malformations and vinyl chloride. Lancet ii:1098.

Ericson, A., B. Kallen, and J. Winberg. 1977. Surveillance of malformations at birth: A comparison of two record systems run in parallel. Int. J. Epidemiol. 6:35-41.

Fiskesjo, G. 1970. The effect of two organic mercury compounds on human leukocytes in vitro. Hereditas 64:142-146.

Gebhart, E. 1977. Pp. 1-192 in Chemische Mutagenese. G. Fischer, Stuttgart-New York.

Hamerton, J.L., N. Canning, M. Ray, and S. Smith. 1975. A cytogenetic survey of 14,069 newborn infants. I, Incidence of chromosome abnormalities. Clin. Genet. 8:223-243.

Hay, A. 1976. Toxic cloud over Seveso. Nature 262:636-638.

Hay, A. 1977a. Seveso: The aftermath. Nature 263:538-540.

Hay, A. 1977b. Seveso solicitude. Nature 267:384-385.

Infante, P.F., J.K. Wagoner, A.J. McMichael, R.J. Waxweiler, and H. Falk. 1976. Genetic risks of vinyl chloride. Lancet i:734-735.

Kimmel, C.A., J.G. Wilson, and H.J. Schumacher. 1971. Studies on metabolism and identification of the causative agent in aspirin teratogenesis in rats. Teratology 4:15-24.

Klingberg, M.A., and C.M. Papier. 1979. Teratoepidemiology. J. Biosoc. Sci. 11:233-258.

Koch, E.R., and F. Varenholt. 1978. Teuflisches Blei. Pp. 132-138 in E.R. Koch and F. Vahrenholt, eds. Seveso ist Überall. Kiepenheuer und Witsch, Köln.

Lenz, W., and K. Knapp. 1962. Thalidomide embryopathy. Arch. Environ. Health 5:100-105.

Lindquist, N.G., and S. Ullberg. 1972. The melanin affinity of chloroquine and chlorpromazine studied by whole body autoradiography. Acta Pharmacol. 31 (Suppl. 2): 1-32.

Marden, P.M., D.W. Smith, and M.J. McDonald. 1964. Congenital anomalies in the newborn infant including minor variations. A study of 4,412 babies by surface examination for anomalies and buccal smear for sex chromatin. J. Pediatr. 64:357.

Matsumoto, H., G. Koya, and T. Takeuchi. 1965. Fetal Minamata disease. A neuropathological study of two cases of intrauterine intoxication by a methyl mercury compound. J. Neuropathol. Exp. Neurol. 24:563-573.

McKusick, V.A. 1975. Pp. 1-837 in Mendelian Inheritance in Man. Johns Hopkins University Press, Baltimore-London.

Merker, H.J. 1977. Considerations on the problem of critical period during the development of limb skeleton. Pp. 179-202 in D. Bergsma and W. Lenz, eds. Morphogenesis and Malformation of the Limb. No. 1 in Birth Defects: Original Article Series, Vol. XIII. Alan R. Liss, Inc., New York.

Nelson, C.J., J.F. Holson, H.G. Green, and D.W. Gaylor. 1979. Retrospective study of the relationship between agricultural use of 2,4,5-T and cleft palate occurrence in Arkansas. Teratology 19:377-383.

Nishimura, H., and T. Tanimura. 1976. General knowledge on teratogenicity of drugs. Pp. 73-92 in H. Nishimura and T. Tanimura, eds. Clinical Aspects of the Teratogenicity of Drugs. Excerpta Medica and American Elsevier Publishing Company, Inc., Amsterdam-Oxford-New York.

Olson, F.C., and E.J. Massaro. 1978. Effects of methyl mercury on murine fetal amino acid uptake, protein synthesis and palate closure. Teratology 16:187-194.

Ramel, C., and J. Magnusson. 1969. Genetic effects of organic mercury compounds. II, Chromosome segregation in *Drosophila melanogaster*. Hereditas 61:231-254.

Rehder, H., L. Sanchioni, F. Cefis, and A. Gropp. 1978. Pathologisch-embryologische Untersuchungen an Abortusfallen im Zusammenhang mit dem Seveso-Ungluck. Schweiz. Med. Wochensch. 108:1617-1625.

Ridgway, L.P., and D.A. Karnofsky. 1952. The effect of metal on the chick embryo: Toxicity and production of abnormalities in development. Ann. N.Y. Acad. Sci. 55: 203-215.

Russell, L.B. 1965. Death and chromosome damage from irradiation of preimplantation stages. Pp. 217ff in G.E.W. Wolstenholme and M. O'Connor, eds. CIBA Foundation Symposium on Preimplanted Stages of Pregnancy.

Ruziczka, P., and Z. Czeizel. 1970. Cytogenetic studies on mid-trimester abortuses. Hum. Genet. 10:273-297.

Slanina, P., S. Ullberg, and L. Hammarstrom. 1973. Distribution and placental transfer of ^{14}C-thiourea and ^{14}C-thiouracil in mice studied by whole-body autoradiography. Acta Pharmacol. 32:358-368.

Stein, Z., M. Susser, D. Warburton, J. Wittes, and J. Kline. 1975. Spontaneous abortion as a screening device. The effect of fetal survival on the incidence of birth defects. Am. J. Epidemiol. 102:275-290.

Stevenson, A.C., H.A. Johnston, M.I.P. Stewart, and D.R. Golding. 1966. Congenital malformations: A report of a study of series of consecutive births in 24 centres. Bull. WHO 34 (Suppl.)1.

Swedish National Board of Health and Welfare. 1977. Pp. 1-11 in Congenital Malformations and Perinatal Death in a Swedish County: A Study of the Relation to Exposure to 2,4,5-T. Socialstyrelsen, Byra.

Tatetsu, S., and M. Harada. 1968. Mental deficiency resulting from intoxication in the prenatal period. Adv. Neurol. Sci. (Tokyo) 12:181-190.

Tuchmann-Duplessis, H. 1975. Pp. 1-267 in Drug Effects in the Fetus. Adis Press, New York.

Vogel, F. 1970. Spontaneous mutation in man. Pp. 16-68 in F. Vogel and G. Rohrborn, eds. Chemical Mutagenesis in Mammals and Man. Springer, Berlin-Heidelberg-New York.

Vogel, F., G. Rohrborn, E. Schleiermacher, and T.M. Schroder, eds. 1969. Pp. 1-80 in Strahlengenetik der Säuger. Thieme, Stuttgart.

Whorton, M.D., and T. H. Milby. 1980. Recovery of testicular function among DBCP workers. J. Occup. Med. 22:177-179.

DISCUSSION

DR. MILLER: In Minamata disease, is the effect on the brain fetotoxic or teratogenic?

DR. REHDER: It is mainly fetotoxic. However, it is difficult to say because brain development takes a long time, and there may be a disturbance in nerve cell migration even in later gestation. Nerve cell death occurs in the brain with Minamata disease. Disturbances of nerve cell migration that would result in nerve cell heterotopias are not actually seen.

DR. MILLER: In radiation injury we know that the susceptible time during gestation is during the period of organogenesis, but the effect is said to be due to cell depletion in the brain. Is that fetotoxic or teratogenic?

DR. REHDER: The early cell depletion is teratogenic, but cell depletion in the more differentiated brain has to be called fetotoxic, even if the result seems to be the same. In one it is hypoplasia, and in the other one it is hypotrophy.

DR. MURPHY: Please elaborate on reports from New Zealand and Arkansas of cleft palate associated with 2,4,5-T exposure.

DR. REHDER: At a conference in Washington last year on 2,4,5-T and dioxin, it was said that the findings are not sufficient to implicate a correlation. Palatine shelf closure disturbances and neural tube closure disturbances are easily diagnosed, and people jump to the conclusion that these are effects of pollution.

DR. MILLER: A cluster of women in a certain industry have had miscarriages. What do you do with a miscarried fetus to make a proper cytogenetic examination? Do you put it directly into formaldehyde?

DR. REHDER: No. Laboratory specialists and gynecologists should be informed and should have all the media they need to preserve tissues and to guarantee that these investigations are done. The studies are very important. People always say the abortion rate has risen due to one or another pollutant, but nobody investigates.

DR. MILLER: What is the simplest procedure to ensure that a fetus is in a state to have its chromosomes examined?

DR. REHDER: Fetuses in spontaneous abortions are often macerated, and it makes no sense to take fetal tissue if you have autolytic changes already. The placenta, however, is always preserved -- if it is still attached to the uterine wall. Amniotic membranes (which you can easily identify) do grow very well, even if the fetus is macerated. You need just a small amount of medium; insert the specimen, and send it to the cytogeneticist. You should also preserve the fetus, of course, for pathoanatomic study.

DR. SUSKIND: Would you comment on the need to be rather careful about how one interprets both fetotoxicity or teratogenicity in relation to the nutritional environment of experimental animals? For example, there is a problem of maintaining an adequate zinc and copper intake, especially with respect to cadmium teratogenicity or fetotoxicity, and even with some other teratogenic agents, such as salicylic acid or the salicylates.

DR. REHDER: Effects other than direct toxicity on the embryos are very important and play a great role. The potential interaction of elements is very important and should be evaluated, perhaps by studying the placenta. Morphologic study of the placenta is a problem, but you will gain experience if you just start to investigate.

DR. MURPHY: Presently there is a great deal of concern among Vietnam veterans as to possible malformations in their children. What is the likelihood of such occurrences long after the exposure period? Might continued testicular change persist and appear in terata some years later?

DR. REHDER: The effect of the alterations results more in the reduction of the fertility rate than in an increase of genetic disease. This finding can be explained by the high cytotoxic effect versus the low susceptibility of germ stem cells to mutagenic effects. The mutation in the offspring occurs in the more differentiated germ cells, which are eliminated after some years. So, the risk decreases.

DR. MURPHY: Is the germinal stem cell generally more susceptible to death than to mutation by chemical agents?

DR. REHDER: The cytotoxic effect predominates; the cell degenerates and does exhibit a mutagenic effect. It does not affect the offspring.

Birth Defects Register in Seveso: A TCDD-Polluted Area

by E. Marni, L. Bisanti, L. Abate, C. Borgna-Pignatti,
G. Maggiore, P. Bruzzi, and E. Montesarchio[1]

A Birth Defects Registry was established in 1978 to screen all infants born to women living near the ICMESA (Industrie Chimiche Meda Societa Anonima) plant where a reaction vessel containing tetrachlorodibenzodioxin (TCDD) exploded in July 1976. All live and stillbirths are reported to the registry by various private and public health sources. A three-level screening program determines the presence of birth defects and their severity. Children are followed through age 6. Although case numbers are small and control groups are difficult to establish, birth defects are being observed more frequently in children born south of the ICMESA plant than elsewhere in Italy. This finding, however, may be due to the more stringent reporting procedures in this region. In addition, many children born in 1972 and used as controls are still classified as "unknown"; followup of this group has proved difficult.

Numerous experimental works on animals (Courtney and Moore, 1971; Greig *et al*., 1973; Murray *et al*., 1977; Neubert *et al*., 1973; Sparschu *et al*., 1971) have demonstrated a cause-effect relationship between 2,3,7,8-tetrachlorodibenzodioxin (TCDD) intake by pregnant females and birth defects in their litters (Table 1). Human exposure, mostly of males, during chemical manufacture or as a consequence of industrial accidents, has been described (Bleiberg *et al*., 1964; Carter *et al*., 1975; Goldman, 1972; International Agency for Research on Cancer, 1978; Jirasek *et al*., 1973, 1974; Kimbrough *et al*., 1977; Poland *et al*., 1971; Sparschu *et al*., 1971; Thiess and Frentzel-Beyne, 1978) on at least 20 occasions (besides the Vietnam war).

---

[1] Drs. Marni, Abate, Borgna-Pignatti, and Maggiore are from the Pediatric Department of the University of Pavia. Drs. Bisanti and Montesarchio are from the Special Bureau for Seveso of the Regione Lombardia. Dr. Bruzzi is from the Institute for the Study and the Therapy of Tumors in Genoa.

TABLE 1

Main Toxic Effects Reported in Litters of Female Animals Treated with TCDD During Pregnancy

Animal	Malformation	Other Problems
Mouse (Courtney and Moore, 1971; Neubert *et al.*, 1973)	Cleft palate Kidney abnormalities	Hemorrhages Edema Interference with development of lymphatic system Fatty infiltration of the liver
Rat (Murray *et al.*, 1977; Sparschu *et al.*, 1971)	Kidney abnormalities	Intestinal hemorrhages Decreased weight Reduced survival Edema
Hamster (Neubert *et al.*, 1973)	Eye Abnormalities	Reduction of fetal weight Hemorrhages Prenatal mortality

The information gathered on malformed infants born in areas contaminated with TCDD is very scanty (Table 2) and poorly documented (Advisory Committee on 2,4,5-T, 1971; Cutting et al., 1970; McQueen et al., 1977; Meselson et al., 1971; Rose and Rose, 1972; Tung et al., 1971).

On July 10, 1976, a reactor at the ICMESA plant in the town of Seveso, Italy, exploded and an 18 km^2 area was exposed to the chemical contaminant dioxin. The monitored area includes 11 municipalities and has a population of 219,358 inhabitants (Table 3).

Italian law requires that public health officials be informed of the birth of any "deformed infant" and of the presence of congenital anomalies of the locomotor system in individuals of any age. These laws, which date back to 1941 and are concerned only with gross deformities, particularly of limbs, clearly appear insufficient for detection of birth defects in normal conditions and are even more inadequate for the present situation. To overcome these limitations, a "Birth Defects Registry" (BDR) has been devised for the area involved in the chemical contamination.

The registry was started in the fall of 1978, with retrospective and prospective studies. It is based on the screening of infants born to women who were living in the area around the ICMESA plant during July 1976.

Figure 1 shows the organization of the BDR. The registry derives information from area hospitals, local health authorities, and pediatric health services. The registry program has two levels of screening; a third level is added when necessary.

TABLE 2

Human Malformations Reported in Populations Exposed to TCDD

Country	Malformation
Vietnam (Rose and Rose. 1972; Tung *et al.*, 1971)	Trimosy 21 Spina bifida Cleft palate
New Zealand (McQueen *et al.*, 1972)	Anaencephalia Spina bifida

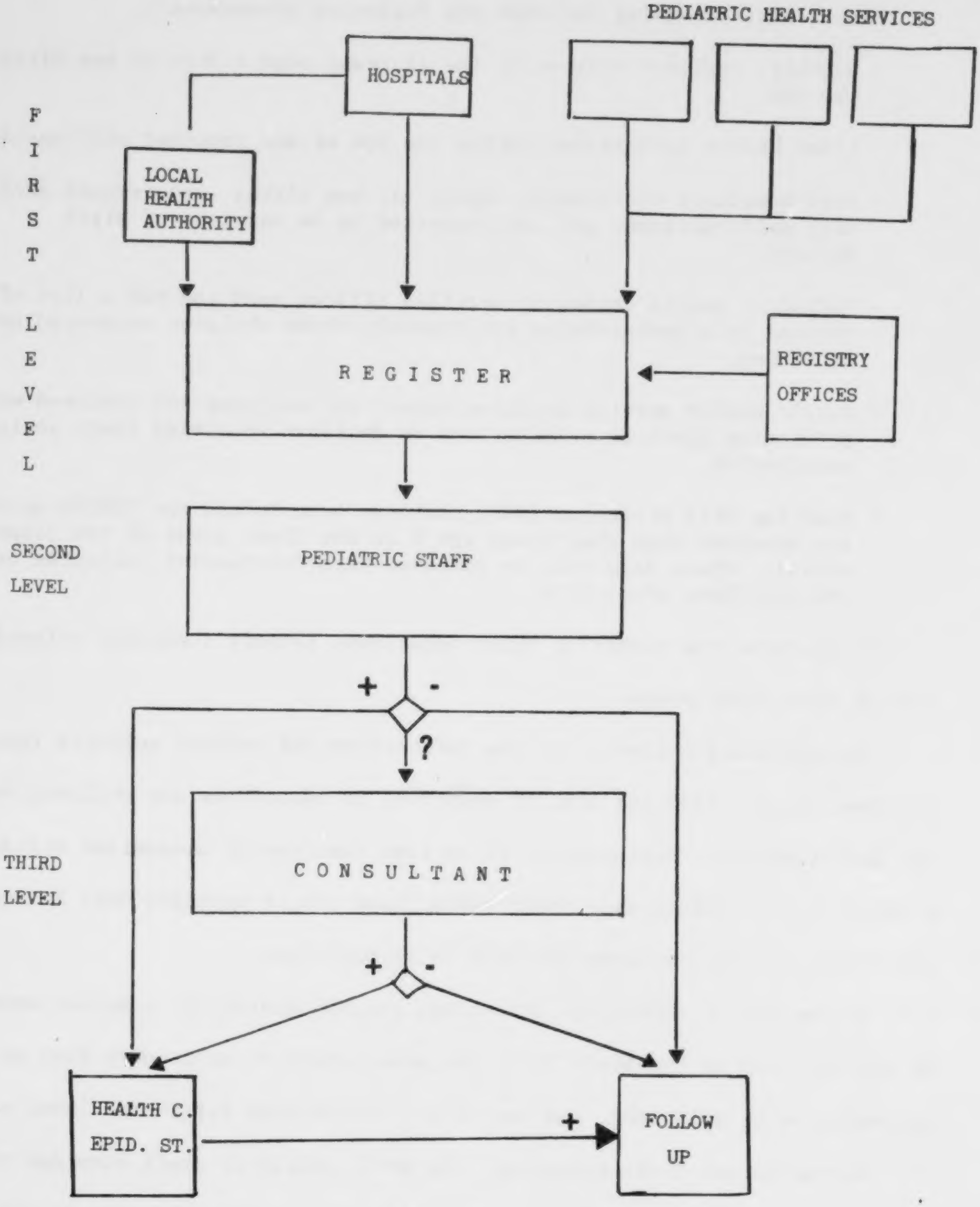
TABLE 3

Population and Area Surface Involved in the Polluting Event

Municipality	Total population	Surface Area X 10,000 sqm	Zone R		Zone B		Zone A	
			pop.%	sur.%	pop.%	sur.%	pop.%	sur.%
Meda	19,668	834	20.4	18.3	---[a]	---[a]	0.3	2.1
Seveso	16,958	734	46.8	37.5	3.7	5.7	4.0	9.5
Cesano M.	33,132	1146	45.1	41.6	8.2	10.2		
Desio	33,021	1479	13.9	29.0	4.1	7.4		
Barlassina	5,635	287	1.3	3.5				
Bovisio	11,170	493	1.5	17.4				
Seregno	36,894	1301						
Lentate	13,140	1399						
Varedo	12,014	484						
Muggio	18,827	547						
Nova M.	18,899	581						
Total	219,358	9285						

---

[a] Zone B is not present in Meda.


PEDIATRIC HEALTH SERVICES
HOSPITALS
LOCAL HEALTH AUTHORITY
FIRST LEVEL
REGISTER
REGISTRY OFFICES
SECOND LEVEL
PEDIATRIC STAFF
+
-
?
THIRD LEVEL
CONSULTANT
+
-
HEALTH C. EPID. ST.
+
FOLLOW UP

ORGANIZATION OF BIRTH DEFECTS REGISTER

FIGURE 1. Organizational model of the birth defects register.

First-level screening includes the following procedures:

- Monthly, registry offices in the 11 towns send a list of new births to the BDR.
- Local health authorities notify the BDR of any reported malformations.
- Area hospitals continually report all new births, any infants admitted with malformations, and any suspected to be carriers of birth defects.
- Pediatric health community services offices send the BDR a list of children checked by a pediatrician and identify those children suspected of being malformed.
- Public health service agencies report any malformation observed while performing physical examinations on children receiving their antipolio vaccination.
- Starting with birthyear 1972, children born before the ICMESA accident are examined when they reach age 6 in the first grade of the elementary school. These data will be compared with information collected on children born after 1976.

To minimize the number of false negatives, largely inclusive criteria are used at this first stage.

The BDR staff collects all the information and unifies multiple reports on the same child. From the list of newborns, it identifies any children who have not been examined. Furthermore, it invites families of unexamined children to a public health clinic near their homes (home visits arranged when indicated) and visits all the children reported to be malformed.

At the end of this first screening, the BDR guarantees complete examinations of all children on the basic list, the preparation of an inquiry list of any children suspected to be malformed, and the recall of children for second-level examinations.

During second-level screening, the BDR's pediatric staff examines the children reported as malformed in the pediatric health clinics or at their homes.

Medical data are recorded as is any possible parental exposure to teratogenic agents. These second-level examinations provide the following conclusions:

Positive: Child diagnosed as being affected by simple or multiple malformations.

Dubious: Additional diagnostic tests or reexamination required, or the clinical picture is difficult to interpret.

Negative: Child free of malformation.

The coordinator of the pediatric staff then lists the positive cases in the registry.

Medical and town records are reviewed and, if necessary, parents are interviewed to establish the cause of death of infants stillborn or dead shortly after birth and to ascertain whether malformations were present.

At the third level, the coordinator of the pediatric staff refers cases with dubious diagnoses to a consultant, whose diagnosis is accepted as definitive.

A cohort study is performed on all newborns and is continued up to age 6 to identify those malformations that only become apparent later. All children are contacted by the registry within the first year of life, between the ages of 1 and 3, and at age 6 when they begin school.

The data presented, updated through September 20, 1979, represent the results of the first year of BDR activity and should be considered preliminary. The children studied were born in 1972, 1976, 1977, 1978, and part of 1979. Table 4 shows the number of live births and malformed infants reported. Table 5 shows the results of the second-level check for the years 1976, 1977, and 1978. The numbers of live births and positive and dubious cases are indicated.

Cases are classified as follows:

Unknown: Subjects for whom no medical information is available. Their percentage varies in different areas and at different times. Furthermore, these subjects may even have a different probability of being malformed.

Known: Children for whom medical information has been supplied by at least one of the sources considered by the registry.

Reported: Subjects suspected of carrying a birth defect.

Reviewed: Children who underwent second-level examination by BDR pediatric staff.

Known, reported, and reviewed subjects include infants stillborn or dead shortly after birth, if the existence of malformations was definitely reported (or excluded) and reviewed.

The secular trend has not yet been analyzed because of the small number of years so far.

Trend analysis of data from elementary school and neonatal screenings (birthyears 1972 versus 1978) will be kept distinct because screening design, observers, and age of population studied are different in the two groups. Unified analysis will be possible for those malformations that can be diagnosed independently of age and observer if the unknown subjects comprise less than 10 percent of the groups.

TABLE 4

Malformed Infants Reported to the Public Health Authority in the Seveso Area Between 1973 and 1978

Year	Live Births	Malformed Infants Reported
1973	3,783	2
1974	3,656	5
1975	3,516	3
1976	3,210	4
1977	2,756	37
1978	2,747	53

TABLE 5

Results of the Second-Level Check: Distribution by Zone and Year of Birth

Zone[a]	Year of birth 1976 (II sem.)	 1977	 1978
Zone A			
Live births	2	4	3
Possible malformations	0	0	0
Dubious diagnoses	0	0	0
Zone B			
Live births	28	67	75
Possible malformations	0	2	6
Dubious diagnoses	0	1	0
Zone R			
Live births	221	375	428
Possible malformations	7	9	18
Dubious diagnoses	2	10	4
Zone A+B+R			
Live births	251	446	506
Possible malformations	7	11	24
Dubious diagnoses	2	11	4
[Zone $\overline{A}+\overline{B}+\overline{R}$][b]			
Live births	1138	2201	2291
Possible malformations	7	45	91
Dubious diagnoses	1	8	15

---

[a] Zones A, B, and R represent areas of high, low, and no soil contamination.

[b] $\overline{A}+\overline{B}+\overline{R}$ represents the area of the 11 municipalities, excluding Zones A, B, and R.

Comparisons will be possible only among populations living within the monitored area due to the difficulties of including in the study a control population outside of the 11 municipalities.

A map of the area at risk from TCDD is being prepared. It will permit identification of different degrees of pollution and epidemiologic comparisons.

The distribution of the positive cases on the area is detailed in the maps in Figure 2 for the years 1972, 1976, 1977, 1978, and 1979. An uneven distribution of cases among municipalities has been observed. This might be due, at least in part, to the small number of birth defect carriers in each municipality. Birth defects are somewhat more frequent south of the ICMESA plant. BDR activity and its results are shown for each year studied in Figure 3.

The malformations identified are indicated in Table 6. Microcephaly cases are still being investigated to establish whether they are primary or secondary effects. Four groups of malformations (spina bifida, microcephaly, Down's syndrome, and hemangioma) appear with particularly high frequency, but their significance has not yet been established.

The data do suggest definitive reasons for the increase in congenital malformations observed over time. The increase in absolute frequency of congenital malformations observed from 1976 to 1978 (Table 4) is probably due to the recordkeeping of the BDR.

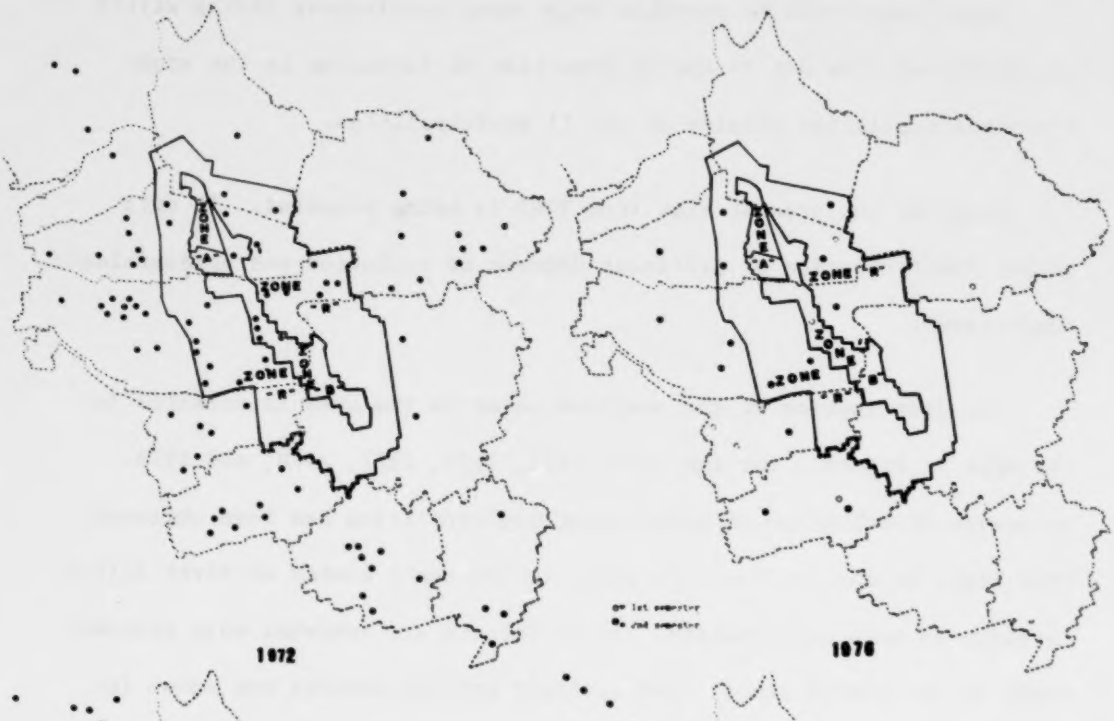
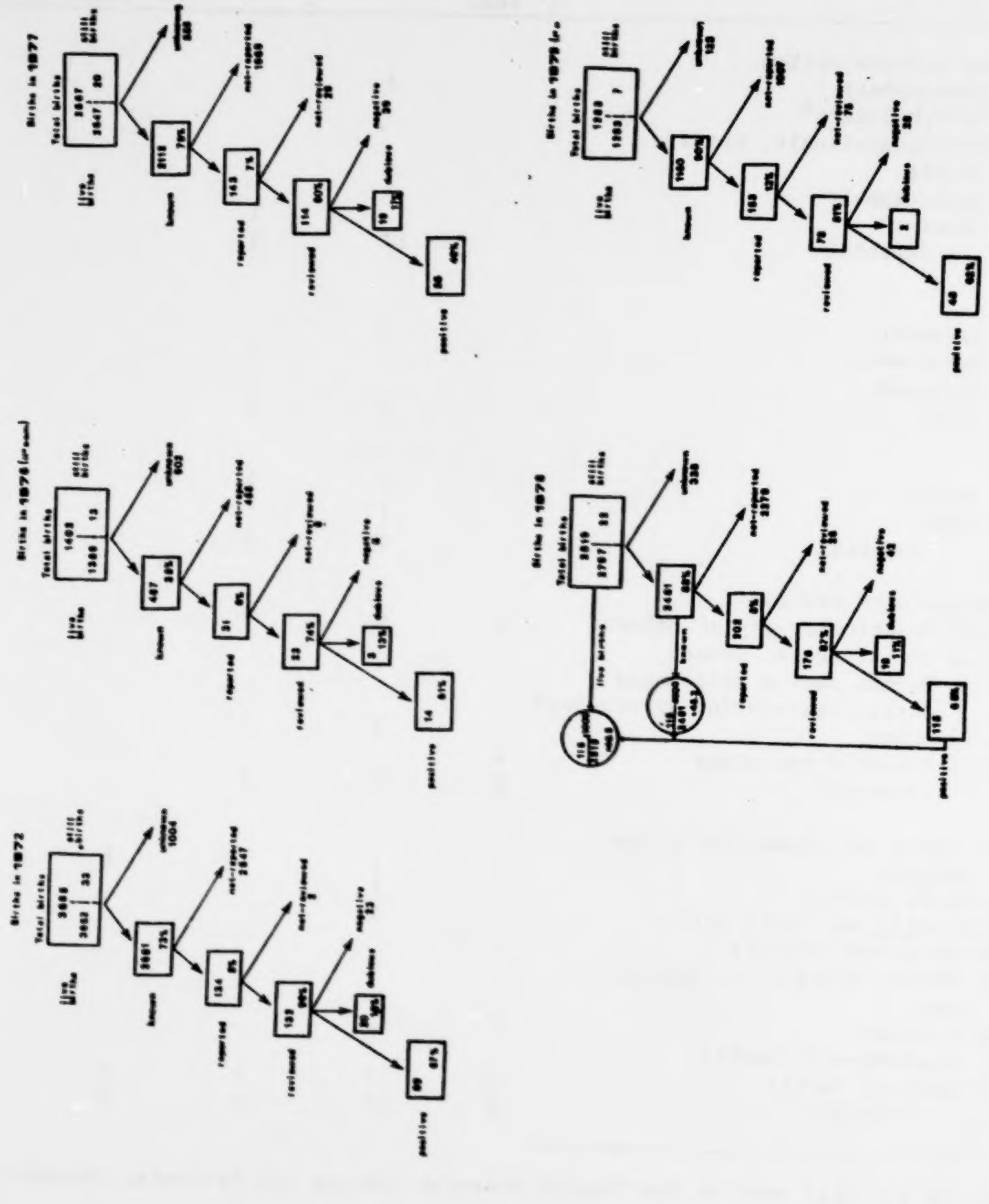

ZONE "A"
ZONE "R"
ZONE "B"
o = 1st semester
• = 2nd semester
1972
1976
1977
1978

FIGURE 2. Maps of the monitored area showing distribution of malformed infants in the 11 municipalities. Zones A,B, and R represent areas of high, low, and no soil contamination as defined on the basis of the chemical analysis performed in the first months after the accident. Birth defect rates are presented for 1978 only, as in that year the monitoring was satisfactory. (• = positive cases carrying one or more malformation; o = malformed infants born before July 10, 1976.



Reproduced from best available copy.

FIGURE 3. Results of the activity of the BDR, through September 1979.

TABLE 6

Positive Cases, Updated to September 1979[a]

Diagnosis (single malformation)	Year of birth				
	1979 1° sem.	1978	1977	1976 2° sem.	1972
Central nervous system					
01 Anencephaly		1			
02 Encephalocele[b]					
03 Meningomyelocele, spina bifida		5	1		
04 Microcephaly		4			
05 Other			1		1
Subtotal		10	2		1
Eye					
08 Cataract		1			
09 Colobomata			1		1
10 Glaucoma					1
11 Other		3	2		3
Ear					
12 Anotia		1	1		
13 Other		1			1
Subtotal		6	4		6
Cardiovascular system					
15/16 Ventricular septal defect	2	6	3		1
17/18 Atrial septal defect					1
20 Patent ductus arteriosus					
21 Aortic coarctation or stenosis					1
22/23 Other		3	1		1
25/26 Fallot's tetralogy	1				3
Subtotal	3	9	4		7
Respiratory and digestive system					
31 Harelip	1	2		1	
32 Cleft palate		1			
33 Harelip and cleft palate		2			
38 Intestinal atresia					1
39 Absent, atresic, or imperforate anus			1		
40/44 Other		2			5
41 Diaphragmatic hernia		2			
43 Inguinal hernia	5	3	4	2	10
Subtotal	6	12	5	3	16

---

[a] Part of the list used by the Italian Research Council for Perinatal Preventive Medicine, Subproject MPP4.

[b] See multiple malformations

TABLE 6 (continued)

Diagnosis (single malformation)	Year of birth				
	1979 1° sem.	1978	1977	1976 2° sem.	1972
Genitourinary system					
48 Extrophia of the bladder			1		
51 Hypospadias	3	5	6	6	10
52 Bilateral cryptorchidism					1
53 Other anomalies/male genitalia		1			
54 Other anomalies/female genitalia				1	
Subtotal	3	6	7	7	11
Bones and joints					
55 Syndactyly	1		2	1	3
56 Polydactyly		4	1		
57 Clubfoot	1	5	1		1
58 Dysplasia of the hip	2	8	8	1	7
60 Other limb defects	1	3			1
61 Osteomuscular abnormalities of head and trunk		1	1		1
63 Chondrodystrophies					2
Subtotal	5	21	13	2	15
Skin					
65 Hemangioma > 0.5 cm diameter (raised or flat in atypical locations)	17	26	8	4	12
64 Other	5	11	2	2	14
Subtotal	22	37	10	6	26
Chromosomal abnormalities					
66/67 Trisomy 21		5	2		4
76 Other			1		
Subtotal		5	3		4
Multiple malformations					
One case for each group of codes	01+02 05+11 +31 11+25 43+58 51+65 55+60 58+64 +65 64+65	02+03 +04+60 04+26 +51+53 18+20 +24 23+54 +56 24+43 43+57 51+55 +60 56+67 64+65	05+08 +23 43+51 43+65 55+60 58+65 64+65	05+13 +52 11+13 64+65	43+51 43+65 60+64

A final diagnosis of "birth defect" has been made in 29% of the "known" infants born in 1976, in 26% of those born in 1977, and in 46% of those born in 1978. However, it is not yet possible to establish whether this increase is due to the ICMESA accident or to the improved reporting system. The rates obtained for 1978 are similar to those reported in the literature (Klingberg and Weatherall, 1979; Takemichi, personal communication, 1979).

There are still many "unknown" children born in 1972; this group includes stillbirths, deaths between the ages of 0 and 6 years, and those who have moved away. The search for these children is incomplete and difficult. Therefore, the real rates for year 1972 could be very close to those observed for 1978.

None of the nine infants born between 1976 and 1978 to women from Zone A (the area with the highest TCDD soil contamination) was malformed. Seven more babies were born in 1979; one of these has a flat angioma on the thigh.

In 1978, 24 infants (of 506 live births, 47%) had birth defects; in the surrounding areas, the percentage of birth defects was 40%. These numbers are small and the detection procedures may have had varying degrees of accuracy.

The data do not show a large increase of congenital malformations attributable to the chemical pollution accident from the ICMESA plant. However, a comparison of these data with those derived from the study of other pathological consequences of TCDD pollution, and a more careful analysis of those malformations frequently observed in the area, are necessary.

REFERENCES

Advisory Committee on 2,4,5-T. 1971. Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency, mimeographed. U.S. Environmental Protection Agency, Washington, DC. 76 pp.

Bleiberg, J., M. Wallen, R. Brodking, and I.L. Applebaum. 1964. Industrially acquired prophyria. Arch. Dermatol. 89:793.

Carter, C.D., R.D. Kimbrough, J.A. Liddle, R.E. Cline, M.M. Zack, Jr., W.F. Barthel, R.E. Koehler, and P.E. Phillips. 1975. Tetrachlorodibenzo: An accidental poisoning episode in horse arenas. Science 188:738.

Courtney, K.D., and J.A. Moore. 1971. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 20:396.

Cutting, R.T., T.H. Phuoc, J.M. Ballo, M.W. Benenson, and C.H. Evans. 1970. Congenital malformations, hydatidiform males and stillbirths in the Republic of Vietnam, 1960-1969. Document No. 903.233. Government Printing Office, Washington, D.C.

Goldman, P.J. 1972. Schwerste akute Chlorakne durch Trichlorophenol-Zeresetzungsprodukte. Arbeitsmed. Sozialmed. Arbeitshyg. 7:12.

Greig, J.B., G. Jones, W.H. Butler, and J.M. Barnes. 1973. Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Cosmet. Toxicol. 11:585.

International Agency for Research on Cancer. 1978. Coordination of Epidemiological Studies on the Long-Term Hazards of Chlorinated Dibenzo-dioxins/Chlorinated Diobenzofurans. Internal Technical Report No. 78/001, draft. World Health Organization, Lyon, France. 48 pp.

Jirasek, L., J. Kalensky, and K. Kubec. 1973. Acne chlorina and porphyria cutanea tarda during the manufacture of herbicides. Cesk. Dermatol. 48(5):306.

Jirasek, L., J. Kalensky, K. Kubec, J. Pazderova, and E. Lukas. 1974. Acne chlorina, prophyria cutanea tarda and other manifestations of general intoxication during the manufacture of herbicides, Part II. Cesk. Dermatol. 49(3):145.

Kimbrough, R.D., C.D. Carter, J.A. Liddle, R.E. Cline, and P.E. Phillips. 1977. Epidemiology and pathology of a tetrachlorodibenzodioxin poisoning episode. Arch. Environ. Health. 28:77.

Klingberg, M.A., and J.A.C. Weatherall. 1979. Epidemiologic methods for detection of teratogens. Vol. 1: Contributions to Epidemiology and Biostatistics. Karger, Basel, Switzerland. 196 pp.

McQueen, E.G., A.M.O. Veale, W.S. Alexander, and M.N. Bates. 1977. 2,4,5-T and human birth defects, mimeographed. New Zealand Department of Health, Division of Public Health. 41 pp.

Meselson, M.S., A.H. Westing, and J.D. Constable. 1971. Background material relevant to presentations at the 1970 annual meeting of the AAAS concerning the Herbicide Assessment Commission for the American Association for the Advancment of Science, mimeographed. Washington, D.C. 47 pp.

Murray, F.J., F.A. Smith, K.D. Nitschke, G.C. Humiston, R.J. Kociba, and B.A. Schwertz. 1977. Three-generation reproduction study of rats ingesting 2,3,7,8-tetrachlorodibenzo-p-dioxin. P. 24 in Proceedings of the 16th Annual Meeting of the American Society for Toxicology, Toronto.

Neubert, D., P. Zens, A. Rothernwallner, and H.J. Merker. 1973. A survey of the embryotoxic effects of TCDD in mammalian species. Environ. Health Perspect. N5:67.

Poland, A.P., D. Smith, G. Metter, and P. Possick. 1971. A health survey of workers in a 2,4-D and 2,4,5-T plant. Arch. Environ. Health 22:316.

Rose, H.A., and S.P.R. Rose. 1972. Chemical spraying as reported by refugees from South Vietnam. Science 177:710.

Sparschu, G.L., F.L. Dunn, and V.K. Rowe. 1971. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Food Cosmet. Toxicol. 9:405.

Thiess, A.M., and R. Frentzel-Beyne. 1978. Mortality study of persons exposed to dioxin after an accident which occurred in the BASF on 13th November 1953. Paper presented at the Fifth International Medichem Congress, 5th-9th September, San Francisco.

Tung, T.T., T.K. Anh, B.Q. Tuyen, D.X. Tra, and N.X. Huyen. 1971. Clinical effects of massive and continuous utilization of defoliants on civilians. Vietnamese Studies 29:57.

DISCUSSION

DR. MILLER: On the basis of animal studies, do you expect a teratogenic effect in humans from exposure to TCDD?

DR. MARNI: A cleft lip, cleft palate, kidney abnormalities, edema, and other anomalies have appeared in mice, rats, hamsters, and some primates. Only a few defects have been noted in humans, and they are not definitely related to TCDD pollution. In Vietnam, two cases of trisomy, a case of palatoschisis, and spina bifida have appeared. Similar observations were recorded in New Zealand.

DR. MILLER: Animal studies for teratogenesis may not relate very well to human experience. Aspirin is a good teratogen in rodent, in which it produces cleft palate and cleft lip, but produces no such effect in humans. Cortisone does the same thing in rodents, but not in humans; and thalidomide does nothing to rodents. At least for those compounds, it is almost as if the effect in humans is opposite to that in animals.

PROF. DARDANONI: On a list of the most frequent malformations, is there any tendency toward one particular type, although the total number of malformations might not clearly be increased? Are certain types of malformation more frequent in certain years?

DR. MARNI: We have few data. We have seen an increase in malformations only in 1978. There may have been an increase in malformations in unknown subjects born in 1976 and 1977. We need to know if three or four types of malformation are at a high incidence with respect to normal data. Also, we need data for past years. We hope to see all or a high percentage of children born between 1972 and 1975. It is a very difficult, though possible, job to find these children who live now not just in our area but, throughout all Italy.

PROF. DARDANONI: An effort is being made to locate these nonrespondents, who sometimes comprise anywhere from 10% to 50% of the sample.

Carcinogenic Effects of Chemical and Physical Agents: Human Observations

Clark W. Heath, Jr.[1]

Study of carcinogenesis in humans is limited by the nature of cancer itself -- its low frequency, its nonspecific clinical nature (lack of specific etiology), the multiple factors involved in its etiology, and long latency periods between exposure and disease appearance. Several specific procedures may help identify relationships between cancer incidence and exposure: a centralized registry would pool cohorts with similar exposures; "marker tumors" could be identified and studied more intensively; and preclinical markers such as chromosomal or mutagenic damage could be established.

The question of carcinogenic effects is ever present in environmental exposure situations. Will a particular exposure increase cancer risk in a specific population? Unfortunately, the question is easy to ask but extraordinarily difficult to answer. There are three approaches to developing an answer:

- o extrapolation from results of animal experiments;
- o inferences from chemical structure or from the known actions of related compounds or similar exposures; and
- o direct observations of humans.

Only the last, observations of humans, can provide final answers. However, the ability to observe is hampered not only by constraints on human experimentation, which dictate reliance on epidemiologic studies, but also by the nature of carcinogenesis itself. The limitations

---

[1] Chronic Diseases Division, Center for Environmental Health, Centers for Disease Control, Atlanta, Ga.

imposed by carcinogenesis are (1) the low frequency with which specific cancers occur, especially in low exposure settings; (2) the nonspecific clinical nature of cancer (that is, specific etiologies cannot be assigned to specific cases); (3) the multifactorial nature of cancer etiology; and (4) the long and variable latency periods that occur between exposures to carcinogens and diagnosis of cancer.

LOW FREQUENCY

When considered collectively over a lifetime, cancer is a common disease. But particular types of cancer occurring over limited time spans are rare. Epidemiologic studies must therefore encompass large populations if they are to measure rates of disease directly. Since risk of cancer generally increases with dose of the carcinogen, it is not surprising that most evidence of human oncogenicity has come from studies of populations with relatively high exposures. In practical terms, this usually means studying exposures in occupational settings (Cole and Goldman, 1975). Prominent examples of occupational carcinogenesis include lung cancer in workers exposed to asbestos; bladder cancer in workers exposed to dyes; leukemia in workers exposed to benzene; and lung, skin, and liver cancers in workers exposed to arsenic. In such instances, high occupational exposures in the past have led to recent increases in cancer incidence. These increases have been of sufficient magnitude to be detected epidemiologically in relatively small cohorts of workers. (An increased incidence of cancer is not, of course, limited to occupational groups, as evidenced by the lung cancer of cigarette smokers and cancers of many sorts in Japanese survivors of the atom bomb.)

Some knowledge of actual dose levels is necessary for any observation of excess cancer in humans to have full impact. Dose-response patterns, if present, provide strong support for concluding that a specific exposure actually caused a particular increase in cancer incidence.

These two elements, therefore--adequate sample size and adequate dose information--are important in any epidemiologic study. The interaction of these two factors is clearly illustrated in the epidemiologic approach currently being pursued to assess the potential carcinogenicity of polybrominated biphenyls (PBB's) in human populations in Michigan (Landrigan *et al.*, 1979). A cohort of some 4,000 persons with relatively high exposure to PBB's is being followed prospectively. Followup will be maintained for at least 15 to 20 years. The group has been observed for 6 years thus far. A major intent of the study is to detect increases in cancer incidence that may occur. Exposure information is incorporated into the study through the subjects' responses to questions concerning PBB exposure and through laboratory measurements of PBB levels in serum. The overall sample size is considered sufficient to detect a significant rise in liver cancer over 5 years. Individual exposure data should eventually make it possible to correlate cancer incidence to PBB levels. For the most part, however, dose levels are low even in this cohort of relatively highly exposed persons. It may be unlikely, therefore, that any increased cancer incidence will be found, given limits in cohort size.

CLINICAL NONSPECIFICITY

Observational tools for cancer epidemiology are at a somewhat primitive stage of development. On the whole, most cancers can be discriminated only by tissue site and cell type, not by particular etiology. By analogy, the main advances in acute infectious disease epidemiology came only after clinical discriminants were supplemented with etiologic tests that isolated viruses and bacteria and measured antibody titers.

This observational handicap is evident everywhere in cancer epidemiology. Assessment of particular etiologies is achieved, not by pinpointing the cause of specific cases, but by adjusting for the relative contributions of competing etiologies, and then measuring any differences that remain.

A simple example is the ongoing study of leukemia incidence among military personnel present at a 1957 U.S. atmospheric nuclear test called "Smoky" (Caldwell *et al.*, 1980). Followup of this cohort of approximately 3,000 men has identified nine cases of leukemia, whereas 3.5 cases might be expected from normal incidence patterns. The study is limited, of course, both by its relatively small size in terms of low expected cancer incidence and by incomplete exposure data. Film badge readings provide some measure of penetrating radiation dose, but no reliable estimates have yet been developed for possible internal exposures from inhalation or ingestion of radioactive materials.

Under these circumstances, it would be quite useful if means were at hand for discriminating between leukemia cases caused by radiation and leukemia cases caused by other exposures. While such discrimination might explain one of the nine cases (a possible variant of chronic lymphocytic leukemia, a form of leukemia not associated with radiation in other studies), the remaining eight cases may or may not be caused by radiation, with five or six related to the nuclear incident.

MULTIFACTORIAL ETIOLOGY

In the absence of clinical discriminants, the multiple causes of cancer make it difficult for cancer epidemiologists to assess relationships between specific exposures and specific patterns of cancer incidence. Analyses must account for competing risk factors while assessing specific etiologic assocations. This process is often not a major difficulty in occupational settings where relatively high exposures can occur. It may also pose no problem for particular types of cancer where the contribution of competing causes may be small (lung cancer and cigarette smoking, for example) or may even be nonexistent (mesothelioma and exposure to asbestos).

The relationship between workplace exposure to vinyl chloride monomer (VCM) and different types of cancer in humans illustrates this point. The very strong association of VCM exposure with hepatic angiosarcoma, a rare tumor with no common competing causes (other causes being arsenic and thorium dioxide), was easily identified and confirmed (Falk _et_ _al_., 1974). In contrast, however, the association of occupational VCM exposure with increase in lung cancer incidence has proved much more difficult to study (Falk _et_ _al_., 1976), despite the much greater frequency of lung cancer compared to hepatic angiosarcoma.

The reason for this difficulty, of course, lies largely in the need to adjust for cigarette smoking as a strong competing risk factor in lung cancer etiology.

Unfortunately, the problems produced by competing risk factors can be expected to be all the greater in situations involving low levels of toxic exposure. Hence, in the Michigan PBB study, it may prove extremely difficult to link observed cancer incidence patterns to actual PBB exposure, given the combination of multiple risk factors and low tissue PBB levels. Conceivably, of course, a rare form of cancer involving relatively few competing variables might easily identify the relationship. For more common tumors, however, such an outcome should not be expected, given the study's limitations in size and the likelihood of strong competing variables.

LONG LATENCY

The problem of long latency imposes perhaps the greatest restriction on human cancer epidemiology, particularly where exposures are recent. In situations where 10 or more years have passed since exposure began, and where sufficient numbers of persons were exposed, retrospective cohort studies can be performed, or case-control studies, if exposures are relatively widespread. Such retrospective approaches, of course, are difficult for the investigator who must reconstruct exposure histories or retrieve complete sets of past disease incidence data.

Prospective studies such as the Michigan PBB project allow one to develop case incidence data as time passes, and they avoid the biases that arise from retrospective risk assessment. But these studies demand patience; they cannot be used to derive quick answers to questions of cancer risk.

CENTRALIZED REGISTRY

Some centralized mechanism is needed whereby persons exposed in specific environmental situations might be registered for long-term followup in a collective or pooled manner. Most individual exposure situations involve too few subjects to justify long-term epidemiologic followup by themselves. However, several similar exposed populations could well provide a sufficient sample size if combined for joint followup. A central registry would maintain records on specific persons exposed in particular situations and permit access at appropriate times for coordinated followup with respect to cancer incidence. To ensure comparability among individual population sets, a standardized data core would have to be established for recording individual features such as age, race, sex, smoking history, and dose information.

MARKER TUMORS

In considering cancer incidence among populations, particular attention might be given to unusual or rare tumor types or to cancers occurring at young ages. Close observation of such unusual "marker" cases might well provide clues to etiology that are obscured by competing risk factors in older subjects or by more common forms of cancer. Epidemiologic studies of tumors such as hepatic angiosarcoma, mesothelioma, and vaginal adenocarcinoma in young women have successfully provided the impetus for this suggestion.

PRECLINICAL MARKERS

New observational tools, which go beyond merely recognizing the clinical occurrence of human cancer and relating such events to environmental exposures are needed. The effectiveness of present-day epidemiologic techniques would be enhanced if data concerning preclinical markers or subclinical biologic damage could be assembled in such a way that eventual cancer occurrence or "cancerproneness" might be predicted accurately. Current interest in markers of chromosomal and mutagenic damage points in this direction and suggests approaches for epidemiologists. The development of such markers, of course, is basically a matter of continued research regarding mechanisms of carcinogenesis.

The effectiveness with which such new knowledge is developed and then applied to epidemiologic studies will ultimately determine how useful observations in humans are in assessing cancer risks arising from potentially toxic environmental exposures.

REFERENCES

Caldwell, G. C., D. B. Kelley, and C. W. Heath, Jr. 1980. Leukemia among participants in military maneuvers at a nuclear bomb test (Smoky): A preliminary report. J. Am. Med. Assoc. 244:1575-1578.

Cole, P., and M. B. Goldman. 1975. Occupation. Pp. 167-183 in J. F. Fraumeni, Jr., ed. Persons at High Risk to Cancer: An Approach to Cancer Etiology and Control. Academic Press, New York.

Falk, H., and R. J. Waxweiler. 1976. Epidemiological studies of vinyl chloride health effects in the United States. Proc. R. Soc. Med. 69:303-306.

Falk, H., J. J. Creech, C. W. Heath, Jr., et al. 1974. Hepatic disease among workers at a vinyl chloride polymerization plant. J. Am. Med. Assoc. 230:59-63.

Landrigan, P. J., K. R. Wilcox, Jr., J. Silva, Jr., et al., 1979. Cohort study of Michigan residents exposed to polybrominated biphenyls: Epidemiologic and immunologic findings. Ann. N.Y. Acad. Sci. 320:284-294.

DISCUSSION

DR. SUSKIND: Some of the major factors that need to be considered are difficult to deal with in constructing an epidemiologic study. Workplace populations in particular present immense problems. What have they been exposed to in addition to the compound under study and how long? Many of these populations have been exposed to several compounds concurrently. What other types of exposure do they have (e.g., drugs, tobacco, and alcohol)? One of the most difficult problems is to derive accurate data about their other exposures prior to the one being studied. And in a retrospective study, subsequent exposures must also be noted and accounted for.

DR. MILLER: You suggested the formation of a central registry for long-term followup of people exposed to certain chemicals, and then said that your own group is studying veterans exposed to low-dose radiation during the Smoky exercise, people exposed to PBB's in Michigan, and vinyl chloride workers. Is the work of the Centers for Disease Control (CDC) being diluted by having to delegate resources to these huge epidemiologic studies that should be spent on the registry for following tumor markers?

DR. HEATH: There is really no question that resources are tied up inappropriately. In the Michigan study, however, the resources are not ours. The major funds are from other parts of the Federal Government. CDC is managing the project, and the work is being carried out by the state health department.

It could be argued, especially in the face of limited resources, that several large followup studies would be enough in terms of long-term followup and that the maintenance of a central registry of smaller events might not be necessary. Such a central registry might largely be a rather dormant project that awakens after 10 years for some linkage.

DR. DARDANONI: Could routinely collected data, such as morbidity data from hospitalized people, be used to monitor certain diseases in the exposed population?

DR. HEATH: That kind of surveillance program for environmentally caused diseases, which are nonspecific, becomes a much larger task than initially expected. There is more reason to set up wide networks of disease surveillance to serve a particular exposure problem.

Experimental Studies on Carcinogenic Effects of TCDD

Giuseppe Della Porta, Maria I. Culnaghi, and Tommaso A. Dragani[1]

The possible carcinogenic activity of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) has been studied in long term experiments in which the compound was administered orally to Sprague-Dawley rats and Swiss mice. Both studies indicated that there were carcinogenic effects. In the rat, an increased incidence of some tumor types and a decreased incidence of others were observed only at the highest dosage level (0.1 μg/kg/day in the diet for 2 years). In the mouse, an increased incidence of liver tumors was noted after weekly doses of 0.7 μg/kg were administered by gavage for 1 year. Three different experiments in (C57BL/6JDp x C3Hf/Dp)F_1 and (C57BL/6JDp x BALB/cLacDp)F_1 mice are in initial stages. In the first experiment, 10-day old mice are receiving 5 intraperitoneal doses of 1, 30, and 60 g/kg once weekly and then observed for 15 months. In the second experiment, female mice are receiving 10 μg/kg by gavage on days 16-18 of pregnancy, and their progeny are observed for 15 months. In the third experiment, 6-week-old mice are receiving weekly doses of 2.5 and 5 μg/kg by gavage for 1 year and then observed for an additional year.

Carcinogenesis was certainly not among the high priority health problems Italian authorities and the scientific community faced soon after the Seveso accident. Certainly, acute and subacute toxicity had to be investigated first; a late effect such as cancer induction had to be studied later.

At the time of the Seveso accident, several years after somewhat similar accidents around the world, practically nothing was known of the carcinogenic potential of 2,3,7,8-tetrachlorodibenzodioxin (TCDD). In the only published experiment, Innes _et al_. (1969) used 2,4,5-trichlorophenoxyacetic acid contaminated with a level of TCDD sufficient to supply 0.27 μg of TCDD/kg/day to mice and obtained negative results.

---

[1] Division of Experimental Oncology A, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.

A few experiments were underway in the United States and in Hungary, but no data were yet available.

The Van Miller report (1977) of a 1.5 to 2-year feeding experiment in SpragueDawley rats showed some indication of carcinogenicity. Unfortunately, the experiment was conducted on only 10 males per dosage level, and no formal evaluation of the significance of its results was possible, particularly because in most treated groups a few single tumors of different types were observed. However, the four lung tumors, the four neoplastic nodules of the liver, and the two cholangiocarcinomas observed in animals receiving the highest dosage level could not be dismissed easily. The highest level was roughly equivalent to 0.3 μg/kg body weight per day. Interim reports of other experiments were also indicating carcinogenicity.

Meanwhile, it had become clear that TCDD was going to remain in the Seveso area and that not only the initial high-dose exposure but also a long-term low-level exposure might occur and involve a large population. Carcinogenicity was therefore considered more seriously and epidemiologic and additional experimental studies were believed necessary.

Toward the end of 1977, our institute was asked to prepare a study of carcinogenicity. One major obstacle was the location of a suitable facility for conducting the experiment. Facilities for long-term studies are limited in Italy, especially sites for conducting a dangerous experiment under some sort of GLP (good laboratory practice) conditions.

In addition, the Seveso accident had created a psychologic antipathy in the general population toward experiments with TCDD.

Since other pharmacologic studies were foreseen, it was decided that three small laboratory facilities would be prepared and equipped for TCDD experiments within the boundaries of the contaminating factory. These rather small laboratories are now located in plastic wagons with air-conditioning and absolute filters. Strict operational rules were established to protect the few people allowed to enter the restricted area. Moreover, it was decided to limit the TCDD load at any given time. The laboratories were ready by June 1978, and went into full operation a few months later.

Preliminary reports of Kociba *et al*. (1978) and Toth *et al*. (1979) were then available. Both the Van Miller *et al*. (1977) and Kociba *et al*. (1978) experiments were performed on Sprague-Dawley rats by feeding them TCDD in the diet continuously; the Toth *et al*. (1979) experiments in Hungary were performed on outbred Swiss mice given TCDD by stomach intubation.

In our study only mice were used, partly because of space limitations. Two F_1 hybrids of inbred strains were selected. The hybrid $B6C3F_1$ (C57BL/6JDp x C3Hf/Dp) has been widely used in the United States and carries from its parental C3H strain the propensity toward a fairly high spontaneous incidence of liver cell tumors. The $B6CF_1$ hybrid (C57BL/6JDp x BALB/cLacDp) carries a susceptibility to leukemogenesis from the C57BL strain and to lung carcinogenesis from the BALB/c strain. The hybrids were also selected because of their strength and longevity relative to those of their parental strains.

For better control of contamination in the laboratory, it was decided not to mix TCDD with the diet. Thus, in the long-term assay, TCDD was given by gavage, dissolved first in a small amount of acetone and then in olive oil to a volume of 0.1 ml/10 g body weight per administration. Treatment was administered once a week to assure a sufficiently continuous exposure relative to the retention of TCDD.

Only $B6C3F_1$ were used for the long-term assay. One untreated control group of 50 males and 50 females is kept in the animal quarters of the Cancer Institute in Milan. A vehicle control of similar size is in the Seveso laboratory. There are two TCDD-treated groups of 50 males and 50 females each.

The treatment was started when the animals were 6 weeks old and will continue for a year. The animals will be observed until the end of their second year, at which time the experiment will be terminated and all survivors will be examined pathologically.

Selection of dosage level was not easy since there was no way to perform a proper 90-day study. In addition, this particular study had to be restricted to two levels of exposure because of the limitation of the facilities. Thus, it was decided to keep both levels in the high-dose zone. Toth et al. (1979) had encountered excess mortality at weekly oral doses of 7 μg/kg body weight. As a consequence, the dosage had been lowered to 0.7 μg/kg body weight. On the other hand, Kociba et al. (1978), working with rats that had an almost 10-fold lower LD_{50} than the mouse, had observed a somewhat high mortality at a dosage level of 0.1 μg/kg body weight per day. Thus, for this study it was decided to use 5 μg/kg/week as the high dose and 2.5 μg/kg/week for the low dose.

The experiment was started at two successive times, and groups of 15 mice of each sex are now approaching the end of treatment. The test animals have suffered low mortality. A growth curve of these initial groups shows a toxic effect in the high-dose males. At 20 weeks of age and after 14 weeks of treatment, these animals already exhibited a significant weight loss compared to the control animals. The low-dose group started to lose weight after 10 weeks. Later on, animals in both groups weighed considerably less than did the controls. The difference was much less evident in the females and involved only those receiving the highest dosage.

Two more studies were initiated. In these, each test group consists of 50 males and 50 females of the two hybrids, B6C3F$_1$ and B6CF$_1$. In the first study, TCDD was given intraperitoneally, starting at 10 days of age, once a week for 5 weeks. These animals will be observed for 1.5 years. This schedule should cover the period of maximal susceptibility to carcinogenesis previously demonstrated with various carcinogens in similar experiments.

This experiment includes one vehicle control group and one group treated with TCDD at 1 μg/kg body weight, five times once weekly, a treatment that did not exert any apparent toxic effect. Mortality among the suckling mice and at the end of the treatment period was within control levels for both hybrids. The growth curves showed no difference between the treated groups and the controls.

To select higher doses for the same schedule of treatment, a small preliminary experiment has been conducted. Results have shown a steep curve of mortality in animals receiving TCDD from 100 μg to 50 μg/kg of body weight five times. At 100 μg, all mice died before the end of treatment, whereas at 50 μg, there was no mortality, but there was a decrease in the mean body weight gain relative to controls. Therefore, we have selected 30 and 60 μg/kg body weight dose levels for two additional experimental groups.

In the second experiment, a transplacental study, (one dose of 10 μg/kg/body weight was given by gavage near the end of gestation at days 16-18. No other treatment will be administered. The litters will be observed for 1.5 years.

Currently, almost 4 years after the Seveso explosion, the implications of exposure to TCDD for carcinogenicity are still unclear, although three studies have already been published. The mouse experiment by Toth _et_ _al_. (1979) was performed in outbred Swiss male mice, which had been given 7 μg of TCDD in sunflower oil by gavage once a week for 1 year. A high mortality rate resulted, precluding a proper evaluation of tumor incidence. The 0.7 μg level produced a significant increase in the incidence of liver tumors in animals. These growths, both benign and malignant, developed in 21 of the 44 treated animals (48%), compared to the matched vehicle controls, of which 7 out of 38 (18%) animals had liver tumors. However, Toth _et_ _al_. also presented the data from three other control groups. One was given carboxymethyl cellulose instead of sunflower oil as the vehicle; the other two were untreated. All three groups had a much higher incidence of liver cell tumors (33%, 33%, and 26%) than was observed in the matched control group. Thus, it is difficult to ascribe the increased incidence of liver tumors to the TCDD. The sunflower oil by itself may have caused a decrease in the spontaneous incidence of liver cell tumors or, more likely in this outbred strain, there is a variation in spontaneous incidence. This experiment seems to indicate hepatocarcinogenicity in the mouse at a relatively low dose, but this hypothesis is not fully substantiated.

The Van Miller *et al.* (1977) experiment provided the first strong indication of carcinogenic activity in Sprague-Dawley rats given TCDD in the diet for 78 weeks, at a dosage level equivalent to 0.3 g/kg body weight per day. The small size of the groups (10 male rats) precludes the evaluation of the results obtained at the smaller dosage levels. It is difficult to ascribe significance to single observations of various different types of tumor with groups of this size.

Kociba *et al.* (1978) also studied Sprague-Dawley rats. In their experiment, groups of 50 males and 50 females received one of three dosage levels 0.001, 0.01, or 0.1 μg/kg body weight daily in the diet for 2 years. The untreated control group included 85 males and 86 females. Both males and females receiving the highest dosage exhibited a significant increase (compared to controls) in the incidence of squamous cell carcinoma of the hard palate and turbinates. Males receiving the highest dosage had an increased incidence of squamous cell carcinoma of the tongue (although two cases were observed among females as well). The incidence of squamous cell carcinoma of the lung increased in the high-dose female group.

The development of squamous carcinomas is an important observation since chloracne is based on a squamous metaplasia. Some of the squamous carcinomas may have developed over squamous metaplasia, involving well-differentiated (perhaps mucin-producing) epithelial cells. In various situations, squamous metaplasia is replacing more sophisticated epithelial elements.

The other group of tumors observed in increased incidence by Kociba _et al_. (1978) were liver cell tumors -- liver nodules and liver carcinomas--but only in the female group. Carcinomas occurred only in the high-dose animals; liver nodules were also observed in those receiving 0.01 μg TCDD. It is difficult to compare these results with the Van Miller _et al_. (1977) data, which are derived only from males.

In the same experiment, Kociba and colleagues showed a decrease of tumors as well; again, the finding occurred in animals receiving the highest dose. There was a significant decrease in the occurrence of endocrine-type tumors, mammary benign tumors, mammary carcinomas, uterine benign tumors, adrenal pheochromocytomas, and pancreatic adenomas in males. This decreased incidence should be further investigated in connection with a possible imbalance produced by a toxic effect of TCDD on endocrine glands directly or indirectly through liver injury.

In an experiment conducted under a National Cancer Institute contract mice and rats were given TCDD by gavage and dermal application, but the final report of this study is not yet available. If results of this experiment confirm the results of previous experiments, particularly regarding target organs, a mechanism of carcinogenesis might be suggested.

Results of other studies may also have implications for TCDD carcinogenicity. First, as demonstrated by Poland and Glover (1979), covalent binding to DNA is extremely low, and mutagenicity testing has not provided convincing results. On the other hand, TCDD is a potent enzyme inducer that may augment the activation of other carcinogens from endogenous or exogenous sources, thereby modifying tumor incidence. The few experiments using TCDD and known carcinogens to study carcinogenesis point to the fact that TCDD may modify carcinogenicity in both directions.

In conclusion, public health officials need to know whether there is sufficient evidence that TCDD is producing tumors in experimental animals; whether the mechanism, direct or indirect, is known; and whether the results can be extrapolated to humans. In addition, the work of epidemiologists will be facilitated or at least the results will provide stronger evidence if target organs can be identified. Not all answers are in as yet. Unfortunately, one of the major lessons is that these experiments should have been performed before the emergency occurred.

REFERENCES

Innes, J.R.M., B.M. Ulland, M.G. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pallotta, R.R. Bates, H.L. Fack, J.J. Gart, M. Klein, I. Mitchell, and J. Peters. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. 42:1101-1114

Kociba, R.J., D.G. Keyes, J.E. Beyer, R.M. Carreon, C.E. Wade, D.A. Dittenber, R.P. Kalnins, L.E. Frauson, C.N. Park, S.D. Barnard, R.A. Hummel, and C.G. Humiston. 1978. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46:279-303.

Poland, A., and E. Glover. 1979. An estimate of the maximum in vivo covalent binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to rat liver protein, ribosomal RNA, and DNA. Cancer Res. 39:3341-3344.

Toth, K., S. Somfai-Relle, J. Sugar, and J. Bence. 1979. Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol-containing dioxin and pure dioxin in Swiss mice. Nature 278:548-549.

Van Miller, J.P., J.J.. Lalich, and J.R. Allen. 1977. Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere 10:625-632.

DISCUSSION

DR. MILLER: Regarding the exposure of mice *in utero*, was the TCDD administered once between the 16th and 18th days of gestation?

DR. DELLA PORTA: Yes.

DR. MILLER: Suppose the critical day for developing tumors was the 14th or 15th days? Why not administer the TCDD in some experiment throughout the pregnancy?

DR. DELLA PORTA: That creates a teratogenesis problem.

DR. MILLER: What about in the last half of pregnancy?

DR. DELLA PORTA: Perhaps TCDD could be administered earlier than the 16th day.

DR. MILLER: Is the TCDD you are administering pure?

DR. DELLA PORTA: Yes.

DR. MILLER: In case there are impurities, should another experiment be conducted using the contamination experienced to see what would happen in humans?

DR. DELLA PORTA: Yes, but this type of experiment is very dangerous for the workers exposed. So, there are severe limitations.

DR. MILLER: Were there any kidney tumors?

DR. DELLA PORTA: No, perhaps one or two observations, but none were statistically significant.

DR. REHDER: Are you going to look for changes such as metaplasia in the respiratory tract or leukoplakic changes within the mouth area?

DR. DELLA PORTA: Yes, we are, including those cases where no tumors occur. The Kociba study does not mention the histopathology in the same areas of animals that had not developed tumors. Perhaps even at the lower dosage levels there may be this kind of pathology.

Adverse Neurologic Effects

Alan M. Goldberg[1]

A study of neurologic effects of chemical contamination poses three major problems: Definition of the questions to be addressed, level and duration of exposure, type of population; current scientific knowledge regarding the chemical's mechanism of action; and an understanding of the effects of a specific chemical on the human nervous system. Exposure to lead and Kepone typify the problems researchers must solve in order to deal with specific polluting events, in terms of current knowledge regarding a chemical itself and the biologic effects of human exposure to it, specifically on nervous tissue.

Response to areawide chemical contamination requires an understanding of the immediate and longer term biologic effects resulting from exposures to both high and low doses of a compound. It is also crucial to understand the interaction between the xenobiotics and the multitude of biological systems potentially at risk.

An investigation of adverse neurologic effects has three components:

1. definition of the questions to be addressed;

2. development of scientific understanding of a chemical's mechanism of action; and

3. understanding the uniqueness of the nervous system as a target for toxic agents.

In addition to biologic considerations, investigators must take into account many variables, such as the characteristics of the exposed population, the ecology, and later consequences of the exposure.

---

[1] Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, Md.

The questions studied and the data collected will be considerably different, depending on whether the population is young or old, male or female, pregnant or nonpregnant. The biological questions must address the toxicity of the compound and its metabolism in humans. These points will be illustrated based on the current literature of exposure to lead and Kepone.

LEAD

Data obtained from the criteria document on lead, published by the World Health Organization (1977), are plotted in Figure 1 and provide a misleading interpretation to relate disease with the levels of lead in blood in children and adults. These data correlate the clinical symptomatology or disease state with blood lead levels and suggest that lead exposure produces similar, if not identical, effects in adults and children. Furthermore, they suggest that children are only slightly more susceptible to the consequences of lead exposure. However, the consequences of lead exposure are very different in the two populations; not only are children more susceptible, the constellation of symptoms is also different. Moreover, lead concentrations in blood only indicate recent exposure and are inadequate and misleading as an indicator of body burden of the metal.

Understanding the consequences of exposure to a chemical agent and its relationship to health effects must be examined as a function of both level and duration of exposure. Figure 2 shows a comparison of a continuous and stable exposure to lead (dotted line) over a

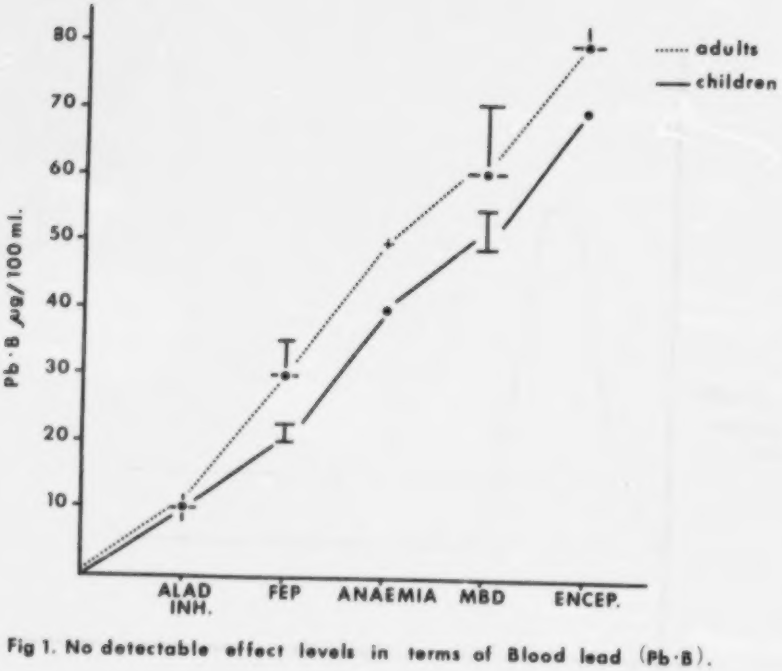


Fig 1. No detectable effect levels in terms of Blood lead (Pb·B).

FIGURE 1. No-detectable-effect levels of lead in blood (Pb.B) of children and adults.

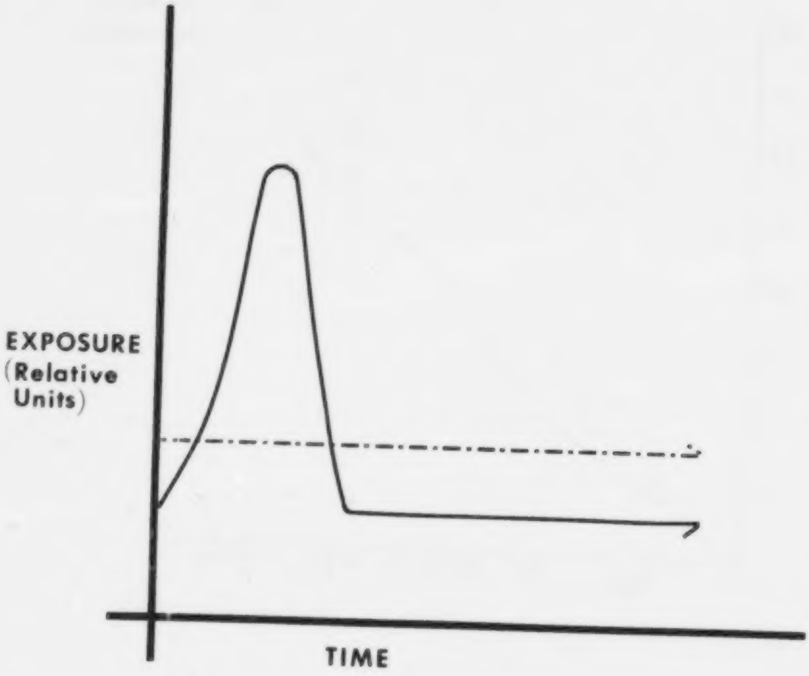


FIGURE 2. Two types of exposure to lead: continuous-equal exposure (dotted line) and an acute high dose exposure (solid line).

prolonged period of time and contrasts it with an acute high dose exposure (solid line). This point is raised to make one aware that the health effects from a high dose acute exposure of lead are different than the health consequences of long term low level exposure to lead.

Persons exposed to various concentrations of lead for varying lengths of time exhibit numerous signs of lead poisoning including drowsiness and fatigue, intellectual deterioration, gastrointestinal distress, anemia, basophilic stippling, kidney damage, and central nervous system effects. The appearance of some of these effects depends on the amount and duration of exposure. Central nervous system effects recorded after low-dose exposures are considerably different from those observed after high-dose exposure (National Academy of Sciences, 1972). Unfortunately, the mechanisms by which the lead produces its effects are known for only two of the symptoms: in anemia, heme synthesis is inhibited, whereas basophilic stippling is the result of a nucleotidase inhibition (Pagalia *et al*., 1975; Valentine *et al*. 1976).

Lead poisoning is an environmental problem of magnitude. In Baltimore, more than 70% of some 30 million private homes and dwellings built before 1940 have surfaces coated with lead-based paint. Public housing in the city is lead-free. Test children (447) from public dwellings showed a mean blood lead level of 16.5 µg/dl. Less than 1% had a lead concentration in blood greater than 30 µg/dl. In 155 children living in private dwellings, the mean lead level was 38 μg/dl. Approximately 78% of these children had a level higher than the 30 μg/dl. Almost 20% had a concentration higher than 50 μg/dl (Chisholm, 1979, personal communication). Clearly, children living in older private dwellings had elevated lead concentrations in blood. These findings are summarized in Table 1.

Settle and Patterson (1980) examined the problem of defining "low-lead." They compared the amount of lead in tuna caught in the deep sea and processed lead-free to that of processed tuna packaged in lead-soldered cans. The deep-sea tuna, which was processed at sea and remained packed in lead-free containers throughout the assay procedure, contained approximately 0.3 ng of lead per gram of fish. The tuna from the lead-soldered cans, however, contained approximately 1,400 ng/g. These results are an indication that the human population is continuously exposed to lead and that the lowest lead exposure level attainable now for the general population is really quite high. Settle and Patterson (1980) also studied bones of Peruvian Indians to calculate their level of "environmental" lead for comparison to current levels. Exposure of the Indians was less than 200 ng/day; humans now are exposed to approximately 29,000 ng/day. These data show the magnitude of the lead-poisoning problem today, both in the United States and throughout the world.

The measures used to quantify exposure to lead have only recently been refined sufficiently to identify body burden. Thus,

TABLE 1

Concentrations of Lead in the Blood of Children Residing in Baltimore, Maryland[a]

Type of Housing	No. of Children Tested	Mean Blood Lead (μg/dl)	Percentage of Children with Blood Lead >30 μg/dl and >50 μg/dl	
			>30 μg/dl	>50 μg/dl
Public (lead-free)	447	16.5	0.7	0
Older private dwellings (>70% with lead-painted surfaces)	155	38	78	19

---

[a] From J. Chisholm, personal communication.

the consequences of low-dose, long-term lead poisoning are just being recognized. Recent studies by Needleman _et_ _al_. (1979) have led to a more refined source from which to measure body burden of lead--the shed tooth. Using lead levels in dentine, these researchers concluded that lead exposure of a magnitude too low to produce clinical symptomatology is associated with neuropsychological deficits that interfere with normal development of children. With various test batteries and scales, these researchers demonstrated a dose-response relationship between dentine lead levels in children and their overall academic performance. Children with high lead levels performed significantly less well (than children with lower lead concentrations) on the Weschler Intelligence Scale and on attentional performance tests as measured by reaction time studies.

Table 2 shows teachers' ratings of children with high and low levels of lead in dentine. "Low" was defined as less than 10 ppm; the term "high" was applied to levels higher than 20 ppm. The table lists the percentage of teachers who indicated negative performances in these children for various characteristics. Children with lead concentrations less than 10 ppm in their teeth received about 10% overall negative responses; children with more than 20 ppm in their teeth received, overall, some 25-30% negative response--almost a threefold increase.

The IQ's (verbal and performance) of the children were matched against the lead concentrations in their dentine (Table 3). Children with low lead levels had IQ's three or four points higher than those

TABLE 2

Teachers' Ratings of Children with High and Low Lead Levels in Dentine[a]

	% negative responses	
Characteristic	<10 ppm	>20 ppm
Distractibility	14	36
Not persistent	9	21
Impulsive	9	25
Easily frustrated	11	25
Daydreamer	15	24
Low performance	8	26

---

[a] From Needleman *et al.*, 1979; reprinted by permission of *The New England Journal of Medicine* 300:689-732, 1979.

TABLE 3

IQ's of Children with High and Low Lead Levels in Dentine[a]

Test	<10 ppm	>20 ppm
Full scale	106.6	102.1
Verbal	103.9	99.3
Performance	108.7	104.9

---

[a] From Needleman *et al.*, 1979; reprinted by permission of *The New England Journal of Medicine* 300:689-732,1979.

of children with elevated lead concentrations. Lead exposure thus produced a significant decrease in IQ's in this population. In these same children, investigators developed a method to measure reaction time, which also measured the distractability of the subjects. The high lead group had a significant increase in reaction time and, thus, a significant decrease in performance.

This compilation of data strongly suggests that there is considerable exposure to low but toxic levels of lead in our environment. The magnitude of the problem is first being defined. Appropriate intervention should be established.

KEPONE

A second environmental contaminant that typifies the problems caused by area-wide chemical pollution is Kepone (chlordecone), a unique chlorinated hydrocarbon, previously used as an insecticide. It was patented in 1952 and registered as a pesticide in 1955. Kepone was produced at two plants in Hopewell, Va. from 1966 to 1974 and at a third plant in the same area during 1974. There is considerable evidence that the chemical found its way into Bailey's Creek, the James River, and finally into aquatic life as early as 1967. The consequences to the health of humans who consumed the contaminated aquatic species, if any, are unknown.

A number of people were exposed to high doses of Kepone during its production in 1974. Exposure to the substance and the effects of that exposure were preventable. When the high-dose exposure to Kepone was first observed in humans, there were no published data pertaining to the consequences of such contamination. The mechanisms by which Kepone exerts biologic effects are just being published now, some 5 years after the exposure, and almost 30 years after the introduction and manufacture of the compound. Such data are basic to devising rational approaches to deal with specific accidents and exposures.

Health effects were not observed in the community neighboring the plant, although contamination was measurable. The acute, high-dose exposure produced major neurologic pathology. One of the most intriguing findings was opsoclonus, a true side-to-side fluttering of the eyes.

Diagnosis of exposure to high levels of Kepone is possible because its cluster of effects is unique. Symptoms include memory difficulties, nervousness, weight loss, joint and chest pain, tremors, and visual difficulties; physical signs involve tremors (rest, postural, and intention), opsoclonus, ataxia, and memory loss. In addition, laboratory tests reveal decreased sperm counts. Although nerve conduction velocities are normal, myelin changes have been observed in nerve biopsies. The consequences of exposure to continuous but low levels of Kepone have not yet been studied.

UNIQUE ASPECTS OF NERVOUS TISSUE

In addressing the problem of neurotoxicity, or the neurological consequences of a chemical exposure, we must understand the unique properties of nervous tissue in order to understand which system or subcomponent of the system is likely to be affected. One of the most unique features of the nervous system is that the nerve cell has its cell body (the energy generating system, the protein synthesis systems, etc.) at one location and an axon, which can be very long, that does not have the capability, e.g., to synthesize protein. Thus, protein must be transported from the cell body to the nerve terminal. A second unique feature of the nervous system is the myelin sheath and it is possible for the myelin to be affected by toxic chemicals. Clearly, if we know that the myelin sheath around the nerve is affected by a toxic chemical we might not yet know how to treat but we will know how to diagnose and evaluate the problem. A third unique feature of the nervous system are the neurotransmitters and related enzymes. The possibility is that chemicals can interact with either the synthesizing enzymes, the integrity of the nerve terminal, the neurotransmitter, or the processes associated with neurotransmission. Clearly, these are specific points at which the nervous system might be susceptible to attack. It is these unique characteristics which, if understood, may reveal how chemicals interact with nervous tissue, thereby allowing us to diagnose, treat, and (hopefully) prevent the damage produced by these chemicals.

In conclusion, appropriate species must be studied to generate the data needed to evaluate the toxicity of chemicals in humans. If an experimental species does not metabolize a compound in the same manner as humans, the information collected is inappropriate for extrapolation to humans. Such species may well incur different biologic consequences of exposure to the parent compound. Second, the basic mechanisms of the interaction of various toxic agents must be known to evaluate the consequences of exposure. For instance, if agents interfere directly with neurotransmission without affecting transmission along the nerve, measurement of nerve conduction velocity would be inappropriate. Knowing which measures are appropriate is dependent on understanding the mechanisms of action of the compound. Last, appropriate tests must be developed to fully assess the damage within the nervous system. The central nervous system is both plastic and redundant. It is possible that chemical damage can result, but it is not easily measured since other parts of the system compensate. We must, therefore, develop techniques to truly assess the consequences to health of such alterations.

REFERENCES

National Academy of Sciences. 1972. Airborne Lead in Perspective. National Academy of Sciences, Washington, D.C. 330 pp.

Needleman, H. L. C. Gunnoe, A. Leviton, R. Reed, H. Peresie, H. Maher, and P. Barrett. 1979. DEficits in psychologic and classroom performance of children with elevated dentine lead levels. N. Engl. J. Med. 300:689-732.

Paglia, D. E., W. N. Valentine, and J. G. Dahlgren. 1975. Effects of low-level lead exposure on pyrimidine 5'-nucleotidase and other erythrocyte enzymes: Possible role of pyrimidine 5'-nucleotidase in the pathogenesis of lead-induced anemia. J. Clin. Invest. 56:1164-1169.

Settle, D. M., and C. C. Patterson. 1980. Lead in albacore: Guide to lead pollution in Americans. Science 207:1167-1176.

Valentine, W. N., D. E. Paglia, K. Fink, and G. Madokoro. 1976. Lead Poisoning. J. Clin. Invest. 58:926-932.

World Health Organization. 1977. Lead, Environmental Health Criteria. World Health Organization, Geneva.

DISCUSSION

DR. MURPHY: What are the significant differences in metabolism of Kepone in rodent and humans? Is the deficiency of the animal model one of metabolism or of target site? The estrogenic actiyity of Kepone was demonstrated many years ago, as were other standard, toxicologic effects. I do not know if opsoclonia was observed in rodents.

DR. GOLDBERG: The strogenic activity of Kepone was demonstrated in both rats and humans, but the metabolic pathway present in humans is lacking in the rodent. Thus, some of the problems caused by Kepone contamination are demonstrable in rodents, and others may not be. Some of the consequences are directly due to the Kepone itself, e.g., the estrogenic activity, while other actions may be due to active metabolites.

DR. MILLER: Where did the Kepone come from in the 1960's when the Life Science Product Corp. was not yet open?

DR. GOLDBERG: Before 1966, Kepone was manufactured by Allied Chemical Corporation in Hopewell, Virginia.

DR. MILLER: The fish with differing Kepone levels at different seasons--did they swim upside down or sideways? Were they obviously ill?

DR. GOLDBERG: No. Nor do the fish kills during 1976 seem related to the presence of Kepone, at least not superficially. The fish in the early spring come from the Chesapeake Bay and are not contaminated. Like the bluefish, they ingest Kepone while feeding as they enter the river, where they remain during the summer months.

Some species, such as oysters and female crabs depurate their Kepone. If they move to clean water, they rid themselves of the compound very quickly. Other species tend to store it--in both edible and nonedible parts.

DR. MILLER: Does Kepone affect a fish's reproductive capacity?

DR. GOLDBERG: That has not been studied.

Exposure to TCDD: Immunologic Effects

Girolamo G. Sirchia and the Group for Immunological Monitoring[1]

Three groups of subjects exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Seveso were monitored: children 3 to 8 years of age; workers at the ICMESA (Industrie Chimice Meda Societa Anonyma) plant where the accident occurred; and soldiers who cordoned off the plant. A battery of immunologic tests were repeated on the same subjects at different times. Results were evaluated by comparing results from (1) exposed and control groups, and (2) exposed chloracne and nonchloracne populations. The children's group was more homogeneous with respect to TCDD exposure. Thus, it was screened more frequently. Findings in that cohort are reported here in more detail. Total serum complement hemolytic activity (CH50) values were significantly higher in exposed children than in controls, for all screenings. Children with chloracne had higher CH50 values than did children without chloracne. Exposed children had higher lymphocytic responses to phytohemagglutinin and pokeweed mitogen than did controls in the first three screenings; later screenings revealed a trend toward higher values, but the increases were not significant. Chloracne incidence did not seem related to these findings. The percentage of children with increased CH50 values, lymphocytes of peripheral blood, and lymphocytic response to lectins was repeatedly higher than expected, especially among children with chloracne. Consistently increased (at two or more screenings) values for tests were shown only for CH50, PBL, and lymphocytic response to lectin, more evident among children with chloracne. A few subjects consistently (at two or more screenings) had values below the lower tolerance limits. Additional investigation is needed, especially with tests more sensitive to slight modifications in values.

---

[1] The following investigators comprise the Group for Immunological Monitoring: Drs. G. Sirchia (Director), V.E.M. Rosso di San Secondo, F. Tedesco, C. Fortis, D. Alcini, A.M. Giovanetti, L. Guazzotti (Centro Trasfusionale e di Immunologia dei Trapianti, Ospedale Policlinico, Milano) (CT); Drs. C. Zanussi (Director), R. Scorza, G. Fabio, P. Bonara, P.L. Meroni, M.G. Sabbadini, M. Vanoli, C. Pettenati (Istituto di Clinica Medica IV, Università di Milano, Ospedale Policlinico, Milano) (CM4); Drs. P. Mocarelli (Director), A. Pessina, P. Brambilla, A. Marocchi (Servizio di Patologia Clinica, Ospedale di Desio, Milano) (OD); Drs. P. Careddu (Director), M. Bardare, F. Corona (Clinica Pediatrica, Università di Milano) (CP); Drs. G. Chiappino (Director), C. Nava, G. Meregalli (Clinica del Lavoro, Università di Milano) (CL); Drs. S. Garattini (Director), F. Spreafico, A. Vecchi, A. Mantovani (Istituto Mario Negri, Milano) (IMN) - only for the first year; and Drs. E. Marubini (Director), S. Milani (Istituto di Biometria e Statistica Medica, Università di Milano).

2,3,7,8-Tetrachlorodibenzo-_p_-dioxin (TCDD) has been reported to cause abnormalities in the immune system in animals. These effects include atrophy of thymus and peripheral nodes (Gupta _et al_., 1973; Vos _et al_., 1973) and decreased resistance bacterial infection (Thigpen _et al_., 1975). Other effects are depression of cell-mediated immune functions, such as lymphocyte response to mitogens (Vos and Moore, 1974; Vos _et al_., 1973), skin graft rejection (Vos and Moore, 1974), and delayed hypersensitivity responses (Moore and Faith, 1976). More recently Faith _et al_. (1978) have reported that exposure to TCDD causes suppression of some T-cell functions (such as delayed hypersensitivity reactions and lymphocyte response to mitogens) but not of others (such as the helper function). It is more difficult to extrapolate this experimental information to humans.

Immunologic data on people exposed to dioxin are scanty, even though dioxin contamination has occurred many times all over the world. One of the major accidental releases of TCDD occurred in Seveso, Italy, on July 10, 1976, when a cloud of vapor containing hundreds of grams of TCDD polluted a densely populated area. Immunological evaluation of individuals believed to have received the greatest exposure has been performed. This paper reports the results of the first 3 years of this monitoring.

Three cohorts were formed for this study. Some 48 children from 3 to 8 years of age, who lived in the most contaminated area, were selected first because they had a (1) possibly higher TCDD:body weight ratio; (2) possibly higher exposure to TCDD because they played in the contaminated soil; and (3) high incidence of chloracne (approximately 50%).

For the second study group, 103 workers at ICMESA, the company where the accident occurred, were selected. Although the accident happened on a Saturday when the plant was closed, most workers were probably highly contaminated because they lived in the polluted area. Moreover, some of them worked at the plant for a few days after the accident. A third group consisted of 75 soldiers who cordoned off the contaminated area. The TCDD exposures in these two groups of adult subjects were less homogeneous than those in the group of children. Thus, this report focuses on the results obtained in children.

Control subjects were not the same persons at each screening, except for approximately 20% of the children, because of compliance problems encountered with this population. Control subjects were apparently healthy people who lived in uncontaminated areas of Greater Milan. Control children were selected from schools in Lissone. Control adults were chemical workers who attended the Clinica del Lavoro in Milan for medical examinations.

The average age of the controls was close to that of exposed subjects within each sex (Table 1). The same procedures were used to collect and analyze blood samples of control and exposed subjects. Thus, any systematic technical errors should affect results from both groups in the same way.

The monitoring protocol included the periodical performance of the following screening tests:

- absolute number of lymphocytes of peripheral blood (PBL);
- titer of antisheep red blood cell antibodies;
- titre of A and B isohemagglutinins;
- serum immunoglobulin concentration (Ig);
- total serum complement hemolytic activity (CH50);
- percentage of erythrocytes (E) rosette-forming cells (E-RFC);

Table 1

Ages, Sex, and Number of Individuals in Exposed and Control Groups[a]

Dates of Screenings	Sex	Median Age (and Range of Ages) and Number (N) of Subjects					
		Children		ICMESA Workers		Soldiers	
		Exposed	Controls	Exposed	Controls	Exposed	Controls
November 1976	F	4.5 (2.5-8) N = 17	5 (3-8.5) N = 15	–	–	–	–
	M	5.5 (2.5-11) N = 27	5.5 (3-8.5) N =27	–	–	–	–
February 1977	F	5 (2.5-8) N = 15	5 (3.5-8) N = 17	–	–	–	–
	M	5.5 (3-13) N = 31	6 (3-12.5) N = 29	–	–	–	–
May 1977	F	5.5 (3-8.5) N = 16	6 (2.5-9) N = 19	– –	– –	– –	– –
	M	5.5 (3-13.5) N = 29	6 (3.5-11) N = 28	– –	– –	– –	– –
December 1977	F	5.5 (3.5-9) N = 17	5.5 (4-9.5) N = 17	– –	– –	– –	– –
	M	6.5(3.5-13.5) N = 28	6.5 (4-9.5) N = 29	– –	– –	– –	– –
May 1978	F	6.5 (4.5-9.5) N = 15	6.5 (4.5-9.5) N = 15	– –	– –	– –	– –
	M	7 (4-14) N = 28	7 (4.5-13) N = 26	– –	– –	– –	– –
October 1978	F	–	–	35 (19-56) N = 4	33.5 (18-50) N = 10	– –	– –
	M	– –	– –	39 (19-65) N = 91	35.5 (23-62) N = 76	– –	– –
May 1979	F	7.5(4.5-10.5) N = 15	8 (6-11) N = 10	– –	– –	– –	– –
	M	7.5 (5-15) N = 28	7.5 (5-14.5) N = 30	– –	– –	– –	– –

Table 1 - continued

February 1979	F	-	-	-	-	-	-
	M	-	-	-	-	22 (20-47) N = 75	22 (19-37) N = 64
		-	-	-	-		
May 1979	F	- -	- -	34 (19-56) N = 3	29.5 (20-41) N = 6	- -	- -
	M	-	-	39 (18-65) N = 76	39 (19-67) N = 80	-	-

- percentage of zymosan-complement rosette-forming cells (ZyC-RFC);
- lymphocyte response to phytohemagglutinin (PHA);
- lymphocyte response to pokeweed mitogen (PWM); and
- lymphocyte response to alloantigens in the mixed lymphocyte culture (MLC).

Each screening lasted approximately 1 month; approximately five exposed and five control subjects were examined each day. The tests were blind, and technicians did not know whether samples had been obtained from exposed or control subjects. Rosette determination and lymphocyte transformation tests were conducted in two different laboratories to allow for evaluation of laboratory performances. Tests were performed as follows:

The absolute number of PBL's was obtained from routine hematologic data (Dacie and Lewis, 1975). To establish the titer of antibodies against sheep erythrocytes and A and B isohemagglutinins, 100 μl of a twofold serial dilution of the test sera, in phosphate-buffered saline, pH 7.4, and containing 0.01 M EDTA, were mixed with 50 μl of a 1% suspension of sheep erythrocytes. Incubation was carried out strictly at 20°C for 1 hour. Agglutination was read microscopically, and the titer of the antibodies was defined as the reciprocal of the highest dilution of the serum that was still able to induce formation of numerous clumps of three or more cells. The same procedure was followed for the titration A and B isohemagglutinins, except that 1% suspensions of A1, B, and O human erythrocytes were used.

Serum immunoglobulins IgG, IgA, and IgM were quantified by the single radial diffusion procedure (Mancini _et al_., 1965), using agar gel plates (Quantiplates Kallestad Laboratories, Chaska, Minn.).

The diameters of the precipitin rings were compared with those of known standard reference preparations. Serum IgE levels were measured by radioimmunoassay (Nye et al., 1975).

The Lachmann and Hobart (1978) technique was used to measure serum complement hemolytic activity. Veronal buffered saline, pH 7.4, mixed with an equal volume of 5% glucose and containing 0.15 mM calcium and 0.5 mM magnesium, was used as diluent. Sheep red blood cells (3×10^8 ml) were sensitized by eight hemolytic doses of rabbit IgM antisheep erythrocytes. An equal number of sera of exposed and control subjects, as well as a pool of sera from 20 healthy blood donors (stored frozen in liquid nitrogen), was tested each day under the same experimental conditions. The results were expressed as CH50 units per milliliter of serum, 1 unit representing the amount of serum required to lyse 50% of sensitized erythrocytes. To compute CH50 units, the observed percentages of lysis at diffeent concentrations of test serum were fitted by a logistic curve according to maximum likelihood principle (following Bliss, 1970).

The test for E-RFC was performed according to Jondal et al. (1972), with slight modifications. Sheep red blood cells (SRBC) were collected in Alsever's solution and stored at 4°C for no longer than 1 week. They were washed five times in buffered salt solution (BSS) and adjusted to a concentration of 10% in BSS containing 20% heat-inactivated fetal bovine serum (FBS) absorbed with SRBC. The lymphocyte suspension was adjusted to a concentration of 2×10^6 cells per milliliter, in BSS containing 20% heat-inactivated FBS absorbed with SRBC. Equal volumes (0.1 ml) of SRBC and lymphocytic suspensions were mixed in round-bottom tubes. These were incubated for 37°C for 15 minutes before centrifugation at 200 g for 10 minutes at room temperature. Tubes were incubated overnight at 4°C. The pellet was gently resuspended with a Pasteur pipette and mounted on glass slides. Some 300 viable cells were counted. Those that bound at least three SRBC's were considered to be E-RFC's.

The test for ZyC-RFC was performed according to Huber and Wigzell (1975), with slight modifications. Plastic tubes (Falcon Plastic, Los Angeles, Calif.) containing a mixture of 25 μl of lymphocyte suspension (4 x 10^6 cells per milliliter) and 25 μl of baker's yeast (0.5 mg/ml), prepared according to Hadding *et al.* (1967) and coated with mouse complement (1 mg of yeast for 0.05 ml of serum), were centrifuged at 90 g for 5 minutes at room temperature and kept in an ice bath for 30 minutes. The pellet was gently resuspended with a Pasteur pipette and mounted with 0.2% trypan blue on glass slides. Some 300 viable cells were counted. Those binding at least three yeast beads were considered to be ZyC-RFC's.

Tests for PHA and PWM stimulations were performed according to Greaves *et al.* (1974) in round-bottom plastic plates (Falcon Plastic) containing 0.5 x 10^5 cells in 0.4 ml of HEPES [4-(2-hydroxyethyl-1-piperazineethanesulfonic acid] vated FBS. All tests were performed in triplicate. PHA (Wellcome purified PHA, Wellcome Italia) and PWM (GIBCO, Bio-Cult Ltd., Scotland) were used at concentrations of 0.1%, 1%, and 5%. Blood lymphocyte preparations were incubated for 3 days. Approximately 12 hours before the end of the culture period, 0.5 Ci of ^{3}H-thymidine (Amersham, England) were added to each well. Cells were harvested on glass fiber filters, and radioactivity was measured in a liquid scintillation counter (Packard Instrument Co., Ill.).

Tests for MLC stimulation were performed according to Helgelsen *et al.* (1973), with slight modifications. Round-bottom plastic plates (Falcon Plastic) contained 0.5 x 10^5 responding and 0.5 x 10^5 stimulating cells per well in 0.4 ml of HEPES (40 mM) buffered RPMI 1640, supplemented with 10% heat-inactivated human AB serum. All tests were performed in triplicate. Stimulating cells (pool of frozen and thawed cells from five unrelated donors) were inactivated by irradiation (4,000 rads). Cultures were incubated for 6 days. Approximately 12 hours before the end of the culture period. 0.5 μCi of ^{3}H-thymidine were added to each well. Cells were harvested on glass fiber filters, and radioactivity was measured in a liquid scintillation counter.

For the chilren, two approaches were devised to obtain surveywide information and to evaluate each subject:

1. Findings from the following groups were compared at each screening of exposed subjects vs controls, and exposed subjects with chloracne vs. exposed subjects without chloracne (Scheffè, 1961).

2. A flowchart of results obtained at different times was set up for each exposed subject and for each immunologic test. Data from each test obtained at each screening, for every single subject, were plotted, together with their reference values. Only data from laboratories that performed at least four screenings were included on the charts.

Since the adults had received only one or two screenings, the approach to their analysis was limited to comparisons of results from exposed and control subjects.

Results of all the tests performed at each screening were evaluated, using analysis of variance techniques. For most of the immunologic tests, the experimental error is positively skewed and its dispersion is related to the mean. Thus, the assumptions underlying the analysis of variance are not fulfilled (Snedecor and Cochran, 1972). Hence, original values were first transformed, as indicated in Table 2, to distribute errors symmetrically and to make them independent of the mean. A 10% level of significance (two tails) was adopted as a threshold to improve the power of the test.

To construct the individual flowcharts, reference values were given in terms of tolerance limits (Guenther, 1977). The limits were computed with a confidence level of $\gamma = 0.95$. Thus, at least a prefixed percentage (P) of the control population would respond within the limits if their tests were performed with the same precision and accuracy as those of the study subjects.

Tolerance limits at each screening (TL_s) were computed as follows,

$$TL_s\ (\gamma, P) = \bar{x} \pm r\ (n, P) \quad u(f, \gamma) . s_s ,$$

where: $\bar{x}$ is the average response of the control group; r is a function of the number (n) of control subjects and of the percentage (P) of the population included between the limits: P values were 80%, 90%, and 98%;

u is a function of the degrees of freedom (f) of s_s and of confidence level γ (values of r and u are reported in Owen, 1962);

s_s is the total standard deviation of the control group and is an estimate of biologic variability "between control subjects" plus technical errors "within day" and "between days."

As n and f increase, r tends toward the percentile of gaussian distribution corresponding to P, and u tends toward 1. However, s_s is particularly high in some tests, such as in the lymphocyte transformation analyses (response to lectins and alloantigens). Therefore, the results obtained for the same subject under the same experimental conditions, but in different days, are highly discordant. The discrepancies are lower among data obtained from subsamples of blood taken from the same subject at the same time and examined separately (Rosso di San Secondo et al., 1979). Table 3 shows details of these variabilities.

The major component of technical variability is "between days." The reference values obtained on different days were widely spread. Thus,

TABLE 2

Transformation of the Data for Symmetry of Distribution and Homogeneity of Variance

Immunologic Test	Expression of Results	Metameter
Lymphocytes	no./μl	Square root
IgG	mg/dl	Logarithmic
IgA	mg/dl	Logarithmic
IgM	mg/dl	Logarithmic
IgE	I.U./ml	Logarithmic
CH50	U/ml	Logarithmic
Anti-A_1 RBC	titer	Logarithmic
Anti-B RBC	titer	Logarithmic
Antisheep RBC	titer	Logarithmic
E-RFC	%	Angular
ZyC-RFC	%	Angular
PHA	c.p.m.	Logarithmic
PWM	c.p.m.	Logarithmic
MLC	c.p.m.	Logarithmic

only gross abnormalities could be detected. To counteract this limitation, the results of lymphocyte transformation tests of exposed subjects were also evaluated in relation to only the values obtained in control subjects tested on the same day in the same experiment. This approach introduced a new source of variability. Different controls were used for each day of screening; but the size of this variability is still lower than that of the variability "between days." Its effects can also be reduced by selecting the control subjects and increasing their number. Table 4 shows how this approach allows the reduction of variabilities and how that reduction is a function of the number of controls (Rosso di San Secondo and Milani, unpublished data).

Tolerance limits for the lymphocyte transformation were obtained for each day of analysis, as follows:

$$TL_d(\gamma, P) = \overline{x}_d \pm r(n_d P)\, u(fp, \gamma)\, s_p$$

where $\overline{x}$ is the average response of the n_d control subjects tested on the d-th day;

s_p is the standard deviation "within day" with f_p degrees of freedom that estimates biologic variability "between control subjects" plus technical errors "within day."

This approach was used only for lymphocyte transformation tests. No information is available on the relative importance of the different components of variability for the other immunologic tests.

Thus, for all the immunologic tests performed, reference values were obtained at each screening from the values of all the control subjects examined at that screening (within-screening tolerance limit = TL_s). For lymphocyte transformation tests, reference values were also calculated daily from the values of the control subjects examined that day (within-day tolerance limit = TL_d).

TABLE 3

Contribution of Each Indicated Source of Variability to Whole Error[a]

Source of variability	MLC	PWM	PHA
Replicates	1.6 (5)	1.8 (1)	1.1 (1)
Subsamples	2.7 (45)	2.8 (8)	2.7 (19)
Days	2.8 (50)	9.5 (91)	5.5 (80)
Whole error	6.2 (100)	10.4 (100)	7.5 (100)

---

[a] Results are expressed as coefficients of variation; figures in parenthesis indicate the percentage of whole error.

For the comparison between exposed and control subjects, 140 F tests of significance were performed at six screenings of 10 tests each. (Lymphocyte response to lectins was tested at three lectin concentrations, and some tests were carried out by two laboratories concurrently.) Another 140 significance tests were performed to compare findings from exposed subjects with and without chloracne. The results can be summarized as follows:

Under the hypothesis that no real difference exists between the two groups of children, it is expected that, by chance, out of 140 tests of significance, 1 or 2 are significant at the 1% level, 7 at the 5% level, and 14 at the 10% level. The observed significant differences are 7, 16, and 25 respectively. Moreover, all the significant differences (except one) are in the same direction (values of exposed children are higher than those of controls).

The highest number of significant differences was observed in the first three screenings (performed between November 1976 and May 1977). In fact, 19 of the 80 tests performed in the first three screenings were significant at the 10% level. Only eight had been expected.

CH50 activity was unique in that exposed subjects had values significantly higher than did controls in all the screenings. CH50 was significantly higher (at the 1% level) only at the first screening in children with chloracne (E^+) than in those without chloracne (E^-). However, if the differences obtained in the remaining five screenings are included, children with chloracne apparently had mean values higher than did children without chloracne. The probability of this occurring by chance is low ($P = 0.031$), indicating that children in screenings 2 to 6 had a $E^+ > E^-$ tendency (Figure 1)

Exposed children showed PHA values significantly higher than did controls at the first screening (performed at the Istituto Mario Negri) and at the second and third screenings at Ospedale di Desio (Figures 2, 3, and 4). If, however, results obtained at the three PHA concentrations at each of the six screenings at Ospedale di Desio are considered together, the averages of the values for exposed subjects are higher than those of controls 16 out of 18 times ($P \simeq 0.001$). A similar tendency is not evident for studies at the Istituto Mario Negri, but only the first three screenings were performed there. Only at the second screening at Ospedale di Desio did children with chloracne differ from children without chloracne significantly. There was no tendency for this difference to occur at any of the other screenings.

Exposed children showed PWM values significantly higher than those of controls at the first screening (at the Istituto Mario Negri) and at the second and third screening at the Ospedale di Desio (Figures 5, 6, and 7). Again, there seems to be a tendency for exposed subjects to have values higher than controls (16 out of 18 times; $P \simeq 0.001$). In this test also, children with chloracne did not seem to differ from children without chloracne.

Exposed subjects showed PBL values higher than did controls at each screening, but the difference was significant only at the second and sixth screenings (Figure 8).

Except for CH50, number of PBL's, and lymphocyte response to lectins, test results in exposed subjects were not clearly different from control values, even though sporadic significant differences have been observed.

The individual flow charts indicated which subjects and which immunologic tests had values outside the tolerance limits. They also suggested a pattern of differences (from the mean of control values) in the 3-year period. Tables were prepared from the charts to highlight single data elements.

The percentage of exposed children with values below the 10th and above the 90th percentiles of control value distribution was calculated for each test at each screening. Analysis of the data indicated that only values for CH50, PBL, and lymphocyte response to PHA and PMW are of interest. of these two percentile groups. In fact, the percentage of subjects with values that exceed the 90th percentile is repeatedly higher than expected for these tests, and is more evident in children with chloracne.

It also became apparent that the calculation of tolerance limits from controls examined within-day (TL_d) affords a more powerful means to detect abnormalities, provided that at least five control subjects are tested at the same time as the exposed subjects (Table 4). In fact, on average, the percentage of subjects with values outside the percentiles (using TL_d) is approximately 30% higher than that obtained using TL_s.

The number of exposed children who, at two or more screenings, had values lower than the 10th, 5th, and 1st percentiles or greater than the 90th, 95th, and 99th percentiles in each of the immunologic tests performed was evaluated.

TABLE 4

Error of Difference in Absolute Value of the Test Subjects, Minus Average Value of Controls Tested Contemporaneously, as a Function of Number of Controls[a]

No. of Controls	MLC	PWM	PHA
1	7.1	8.4	8.1
2	6.4	7.4	7.0
3	6.1	7.0	6.5
4	5.9	6.7	6.3
5	5.8	6.6	6.1
6	5.7	6.4	5.9
7	5.6	6.3	5.8

---

[a] Results expressed as coefficients of variation.

Increased values as reported by the same test, in the same subject, at two or more screenings, were consistently found only for CH50, number of PBL's, and lymphocyte response to lectins, and was more evident in children with chloracne. In fact, seven subjects (four with chloracne) showed values of CH50 higher than the 90th percentile at two or more screenings (expected number = 5.8; 2.6 with chloracne). Of these seven subjects, two (both with chloracne) showed values higher than the 99th percentile (expected numbers, 0.1; 0.03 with chloracne). No subjects had values lower than the 10th percentile.

In the PBL analyses, 11 subjects (8 with chloracne) had values higher than the 90th percentile at two or more screenings expected number same as above. Of these, seven (five with chloracne) showed values higher than the 95th percentile (expected number = 1.5; 0.7 with chloracne).

Considering tolerance limits obtained from TL_d controls, seven children (all with chlorance) had values of lymphocyte response to PHA 5% higher than the 90th percentile at two or more screenings (expected number, 5.8; 2.6 with chloracne). Similar data were recorded when lymphocyte responses to PHA, 0.1%, and PMW, 1% and 5%, were considered. Finally, a few subjects, at two or more screenings and in some tests, had values below the lower tolerance limits. Such results occurred especially for the levels of IgA and IgM (for which one and two subjects, respectively, repeatedly fell below the 1st percentile) and, to a lesser extent, for the titer of antisheep RBC antibodies and for the lymphocyte response to PWM, 0.1% and 1%.

For the groups of ICMESA workers and soldiers, the comparison between exposed vs control subjects at each of the screenings and for the 10 immunological tests gave a number of significant differences compared to those expected by chance.

Has the TCDD released at Seveso caused some transient or permanent alteration to the immune status of exposed people? The study group tried to answer the question by concentrating on results obtained from monitoring children, probably highly exposed to TCDD, for a 3-year period.

Data indicate that exposed subjects showed a CH50 significantly higher than that of controls at each of the six screenings performed. They also had values of lymphocyte response to lectins that were higher than those of the controls at each screening, although significant differences were observed only in the first three screenings. Exposed subjects also tend to have higher PBL values than do controls.

These findings are somewhat surprising because experiments in animals indicate that TCDD has an immunosuppressive effect (Faith *et al.*, 1978; Moore and Faith, 1976; Vos *et al.*, 1973; Vos and Moore, 1974). However, those experiments were performed under different conditions (dose, timing, route of exposure) than the ones discussed in this paper. These variables can influence whether physical and chemical agents, induce either stimulation or suppression, thereby affecting test results. Furthermore, animal data on CH50 are lacking.

Nonetheless, the data from this study must be interpreted with care; every effort was made to obtain the best possible selection of control subjects, but they cannot be considered ideal from an epidemiologic point of view. Secondly, data were not obtained from a specifically designed experiment, but from an observational study, which is exposed to a high risk of erroneous conclusions.

If systematic differences between exposed and control subjects not due to TCDD exposure can be ruled out (as may be suggested by the fact that increased values seem to appear more frequently in children with chloracne than in those without), the mechanism of the increases becomes an interesting subject for speculation. Increased responses of lymphocytes to lectins could result either from a direct stimulating effect of TCDD on lymphocytes or from an indirect action. Vos and Moore (1974) reported that lymphocyte response to lectins *in vitro* is not modified by the presence of TCDD, but there is some evidence (Pandian and Talwar, 1971) that TCDD *in vivo* stimulates the lymphocytic incorporation of thymidine through the release of somatotrophin by the pituitary gland. More recently, however, Vecchi *et al*. (1980) have reported that the *in vitro* response of mouse lymphocytes to Concanavalin A increases significantly in the presence of TCDD, if appropriate amounts of the chemical are used. Alternatively, increased lymphocytic responses to lectins could be the result of the depressive action of TCDD on some suppressor mechanisms, which has been shown to occur for small-dose radiation (Anderson and Lefkovits, 1979).

The increased CH50 hemolytic activity could be explained by the increased production of complement components as well as other "acute phase proteins" by the liver in response to tissue damage by TCDD. If TCDD does cause the modifications observed *in vitro*, one must then determine their clinical importance. Immune reactivity may have increased; however, elevated rates in some tests do not necessarily imply increased immune reactivity. An increase of some indices, such as the level of immunoglobulins, is even found in some congenital immunodeficiencies (World Health Organization Scientific Group on Immunodeficiency, 1979). Moreover, even if the increases observed in this study are the result of increased immune reactivity, immunodepression may follow the stimulation. Thus, the few subjects with decreased IgA, IgM, antisheep RBC antibodies, and PMW values should not be disregarded.

During the 3-year observation period, no particular clinical event besides chloracne seems to have occurred in the exposed children. Thus, it is difficult to correlate *in vitro* and *in vivo* events and to draw conclusions about the clinical value of the data from *in vitro* studies. This information may be obtained only by continuing appropriately planned immunologic and clinical investigations of exposed children for many years.

Present studies indicate several areas requiring modification in future investigations. Some tests used in this investigation show a variability that could obscure slight modifications occurring over a period of time. An *ad hoc* experiment to quantify the principal sources of variability of the most variable tests (such as the lymphocyte transformation analyses) was performed (Owen, 1962; Rosso di San Secondo and Milani, 1979), and the information collected was used to improve the design of the protocol. It is clear, however, that tests for immunologic monitoring must be made more sensitive to slight variations. It is difficult to monitor the immune status of a large number of subjects effectively, i.e., to reconcile effectiveness and feasibility. The protocol in this study seemed the only one possible 3 years ago. A different set of immunologic tests might have provided a deeper insight into the problem. Other tests should be added, including some sophisticated ones, to evaluate T and B lymphocytes and phagocyte function. In addition, this study examined only a selected group of exposed people. It was assumed that, if no abnormality became apparent in the children, then abnormalities would probably not be detected in cohorts that were exposed to lower concentrations.

Regardless of the limitations, the Seveso study has taught at least three major lessons:

1. A permanent investigative group, able to cope with future areawide chemical contamination, should be established.

2. Tests used must detect slight modifications of the immune status of exposed subjects.

3. The clinical significance of the *in vitro* findings must be investigated further.

4. Accidental environmental contaminations are occurring with increasing frequency. Thus, national systems to evaluate the immune status of exposed populations and international cooperation are needed.

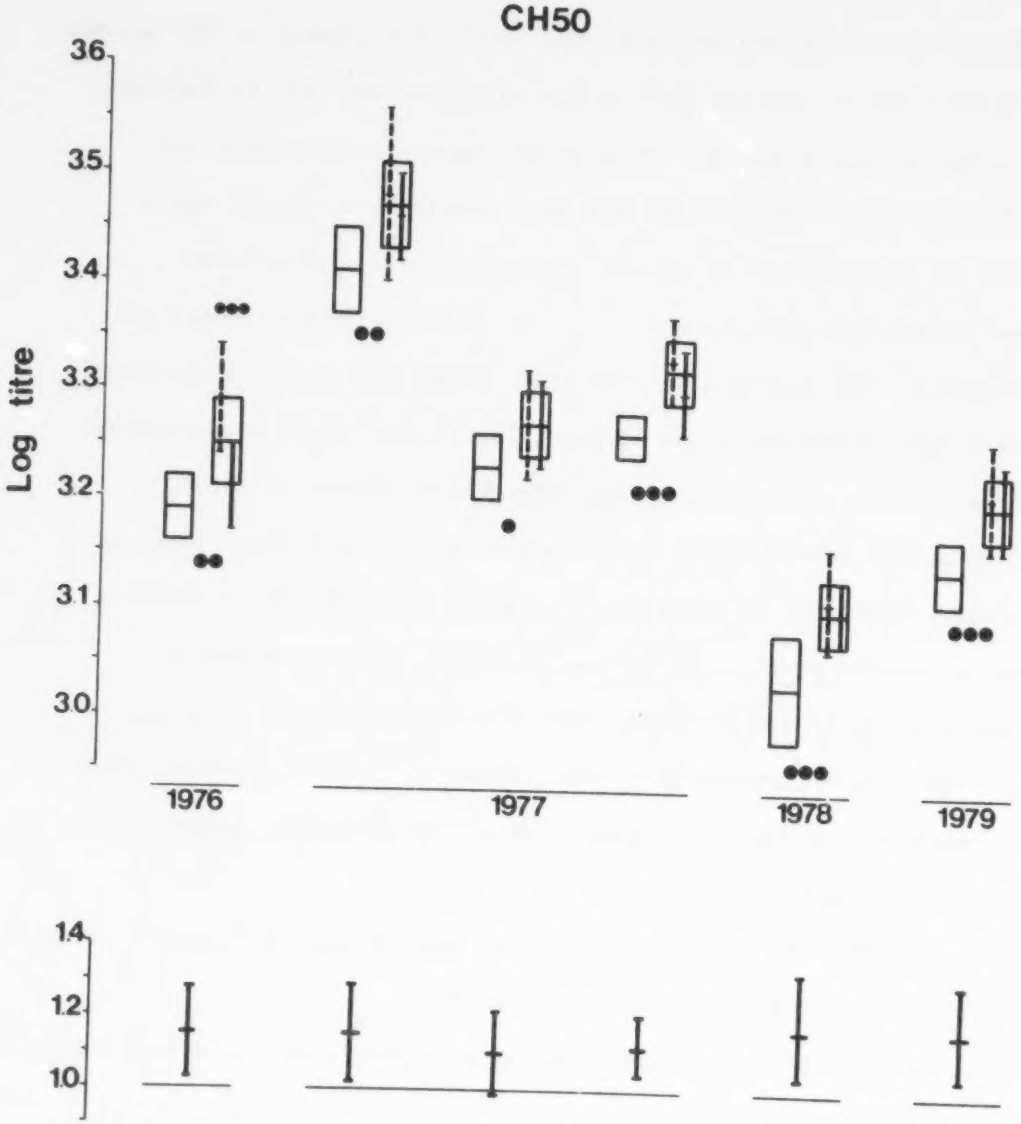


FIGURE 1 - Group of children. Complement activity.

Upper portion of figure: Comparison between control (left-hand box) and exposed children (right-hand box) at each screening. Boxes indicate the mean and the confidence limits for exposed subjects with (interrupted line) and without (continuous line) chloracne. • significant at the 10% level; •• significant at the 5% level; ooo significant at the 1% level.

Lower portion of figure: As above, but control values are indicated as a baseline so that differences from controls occurring in exposed children (vertical lines) are more evident. Ordinates are the ratio of mean value of exposed subjects to mean value of controls with the 95th confidence limits.

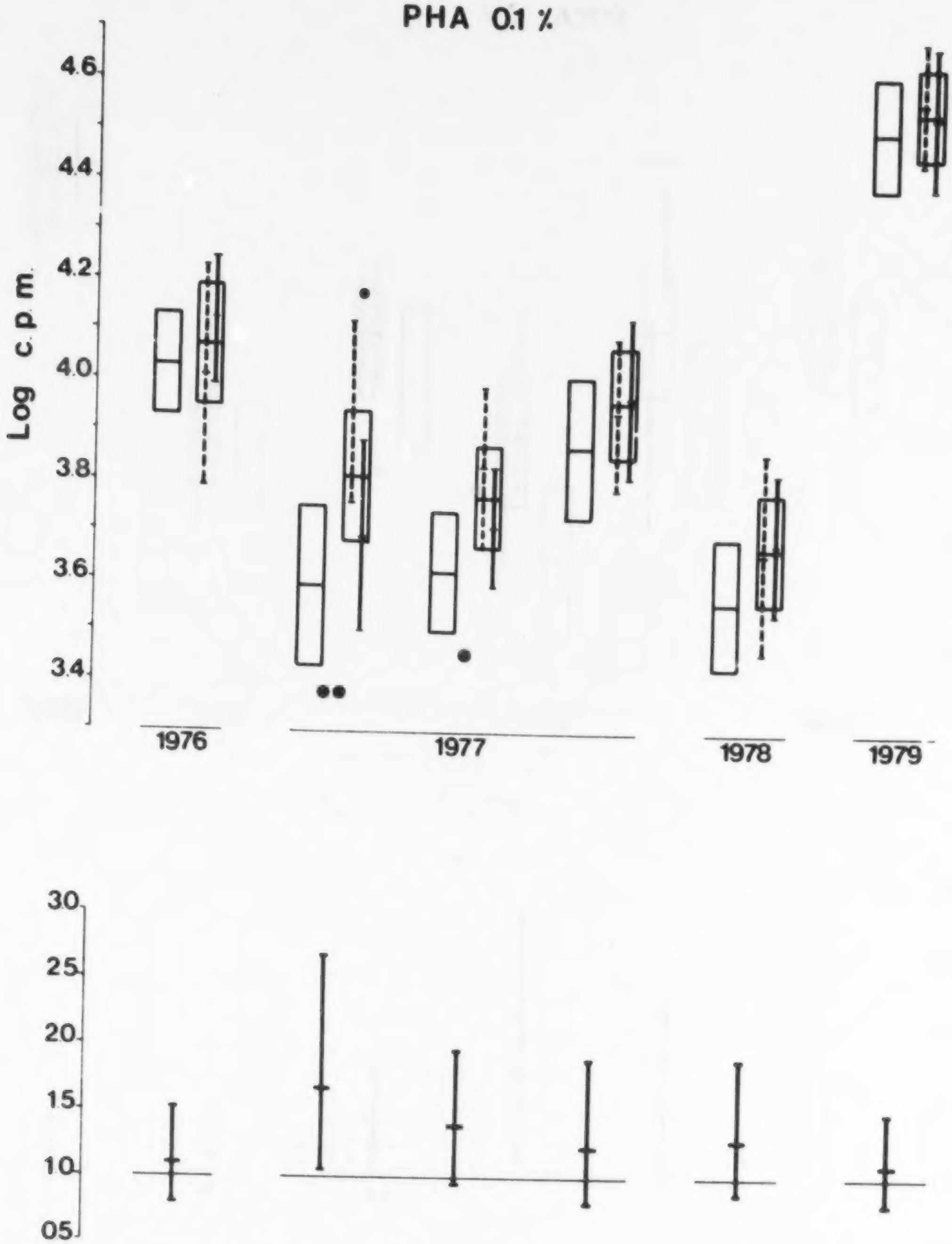


FIGURE 2 - Group of children. Lymphocyte response to PHA 0.1% (performed at Ospedale di Desio).

For the legend, see Figure 1.

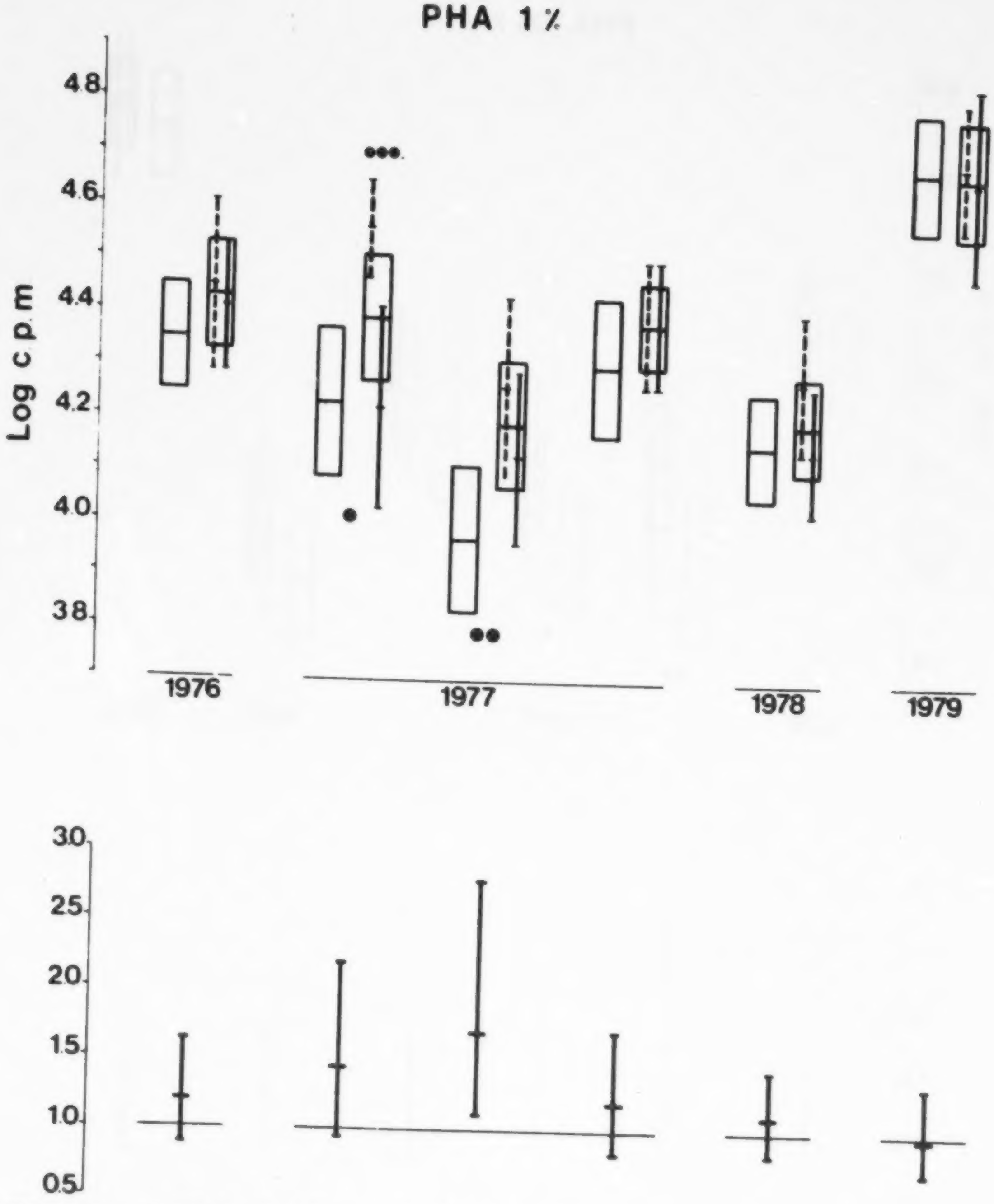


FIGURE 3 - Group of children. Lymphocyte response to PHA 1% (performed at Ospedale di Desio).

For the legend, see Figure 1.

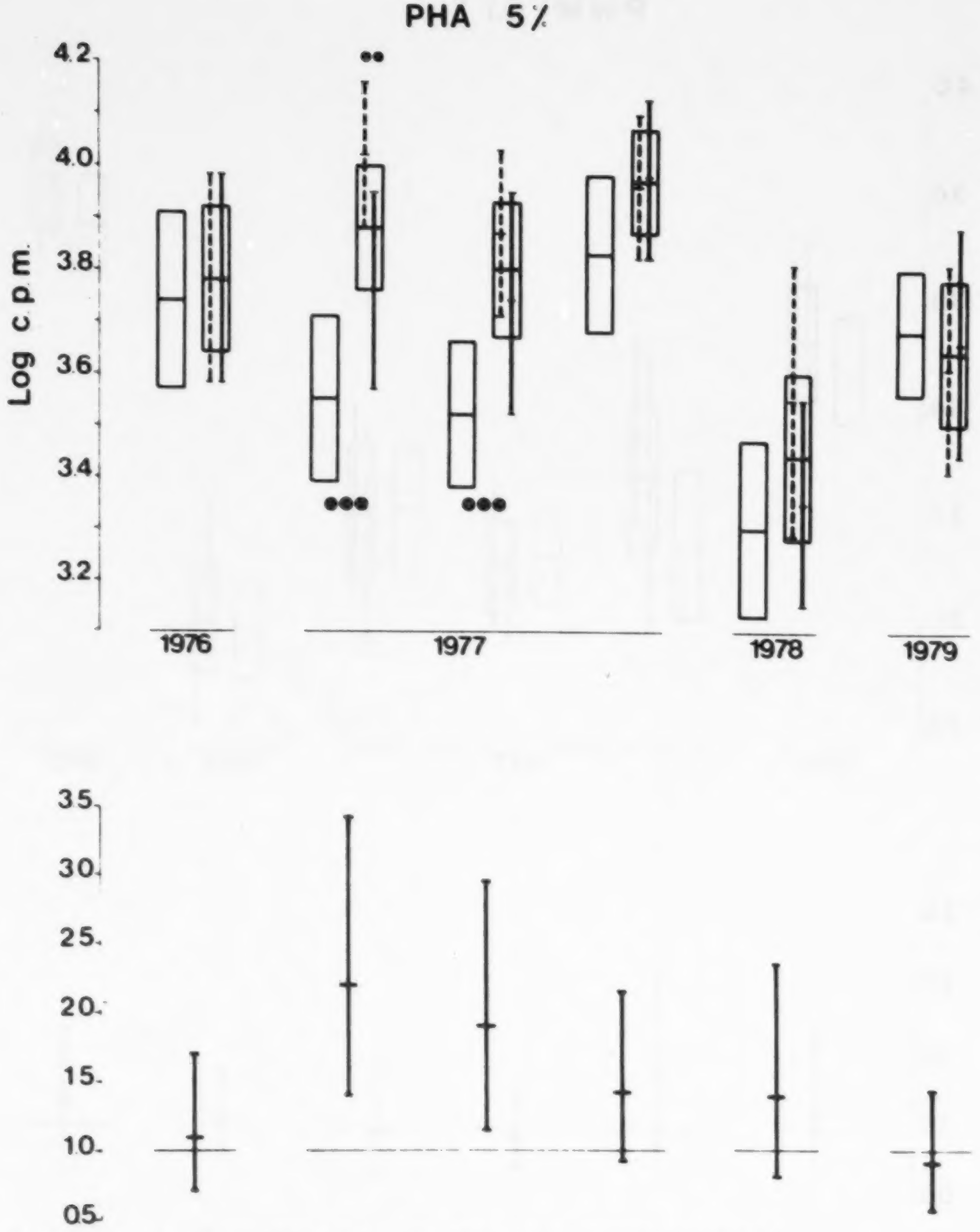


FIGURE 4 - Group of children. Lymphocyte response to PHA 5% (performed at Ospedale di Desio).

For the legend, see Figure 1.

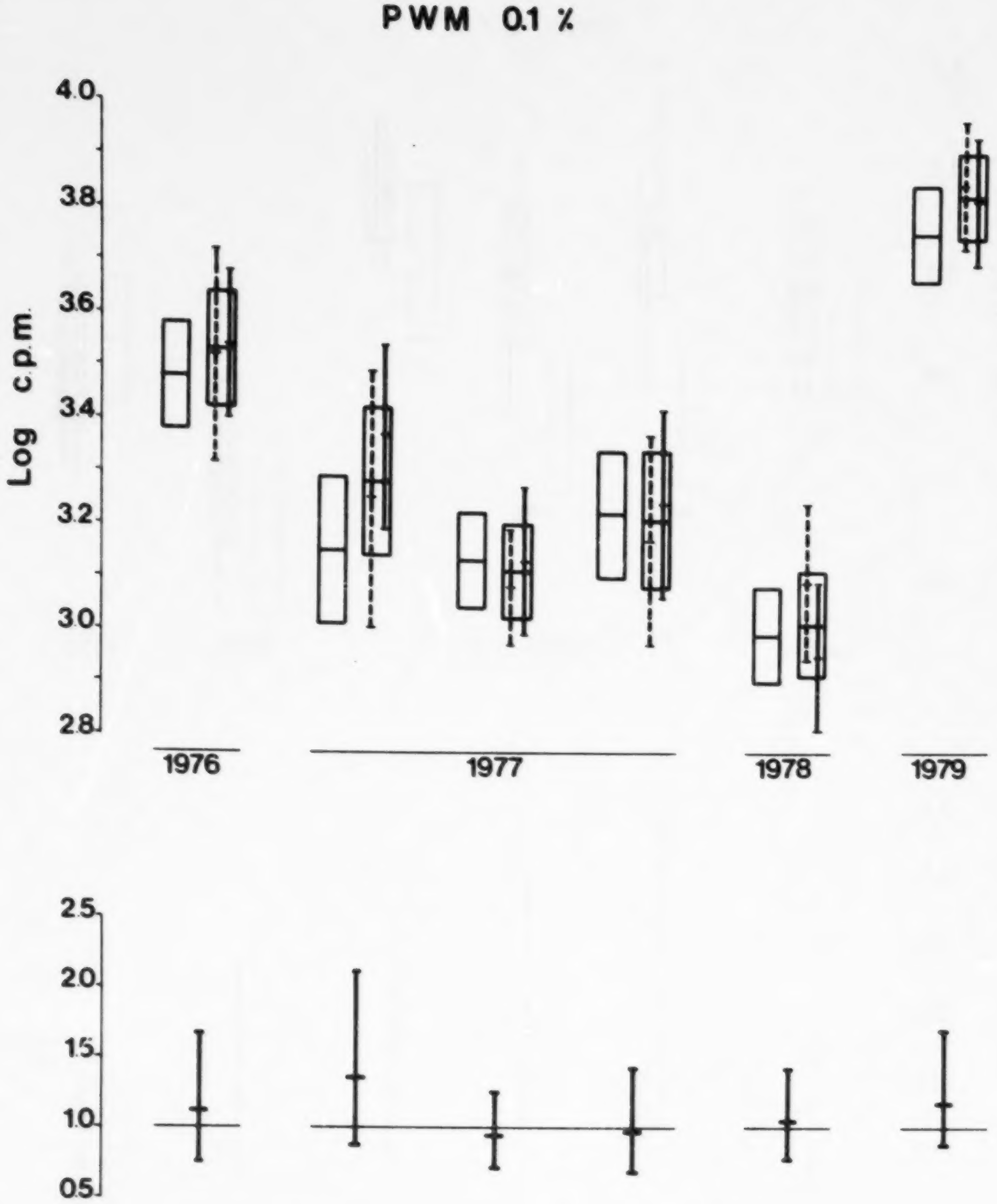


FIGURE 5 - Group of children. Lymphocyte response to PWM 0.1% (performed at Ospedale di Desio).

For the legend, see Figure 1.

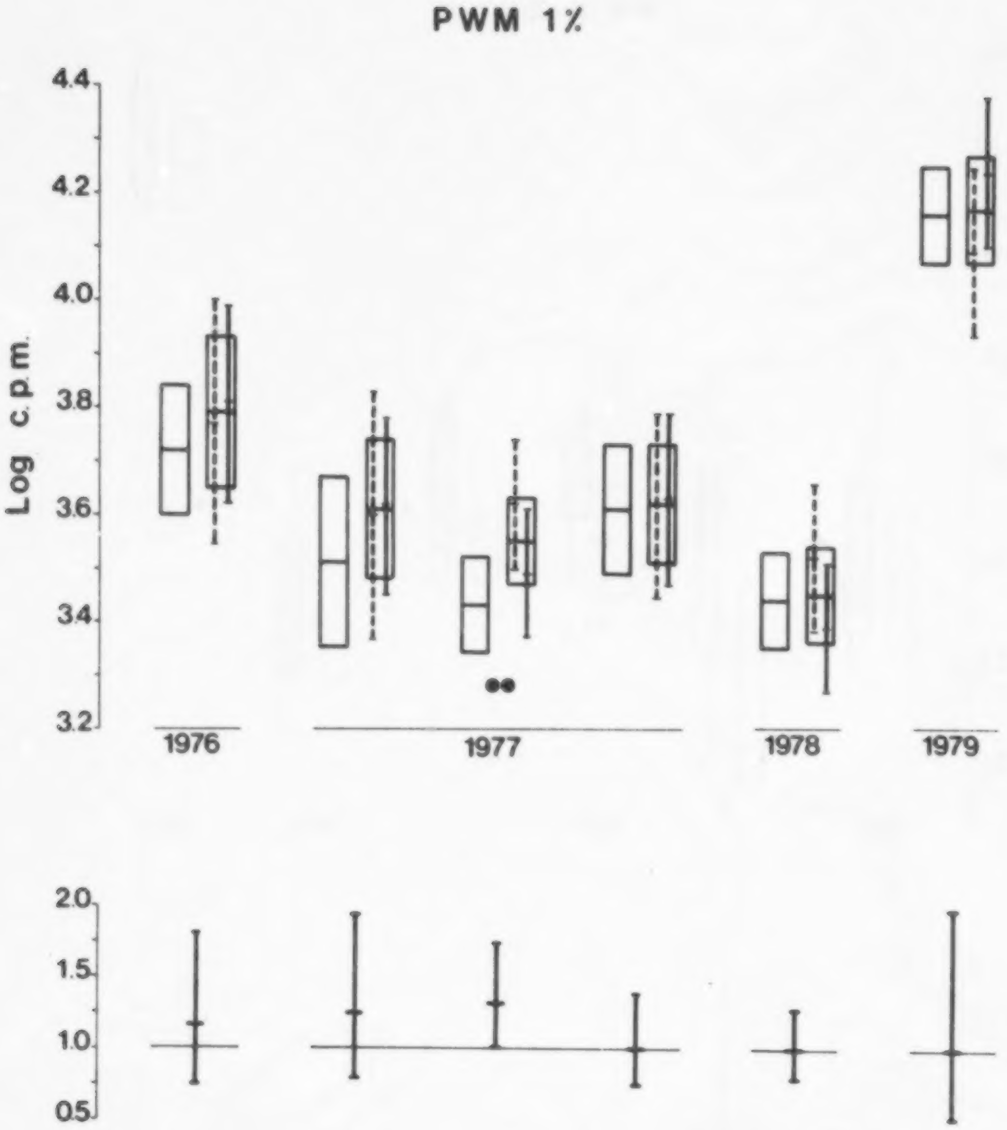


FIGURE 6 - Group of children. Lymphocyte response to PWM 1% (performed at Ospedale di Desio).

For the legend, see Figure 1.

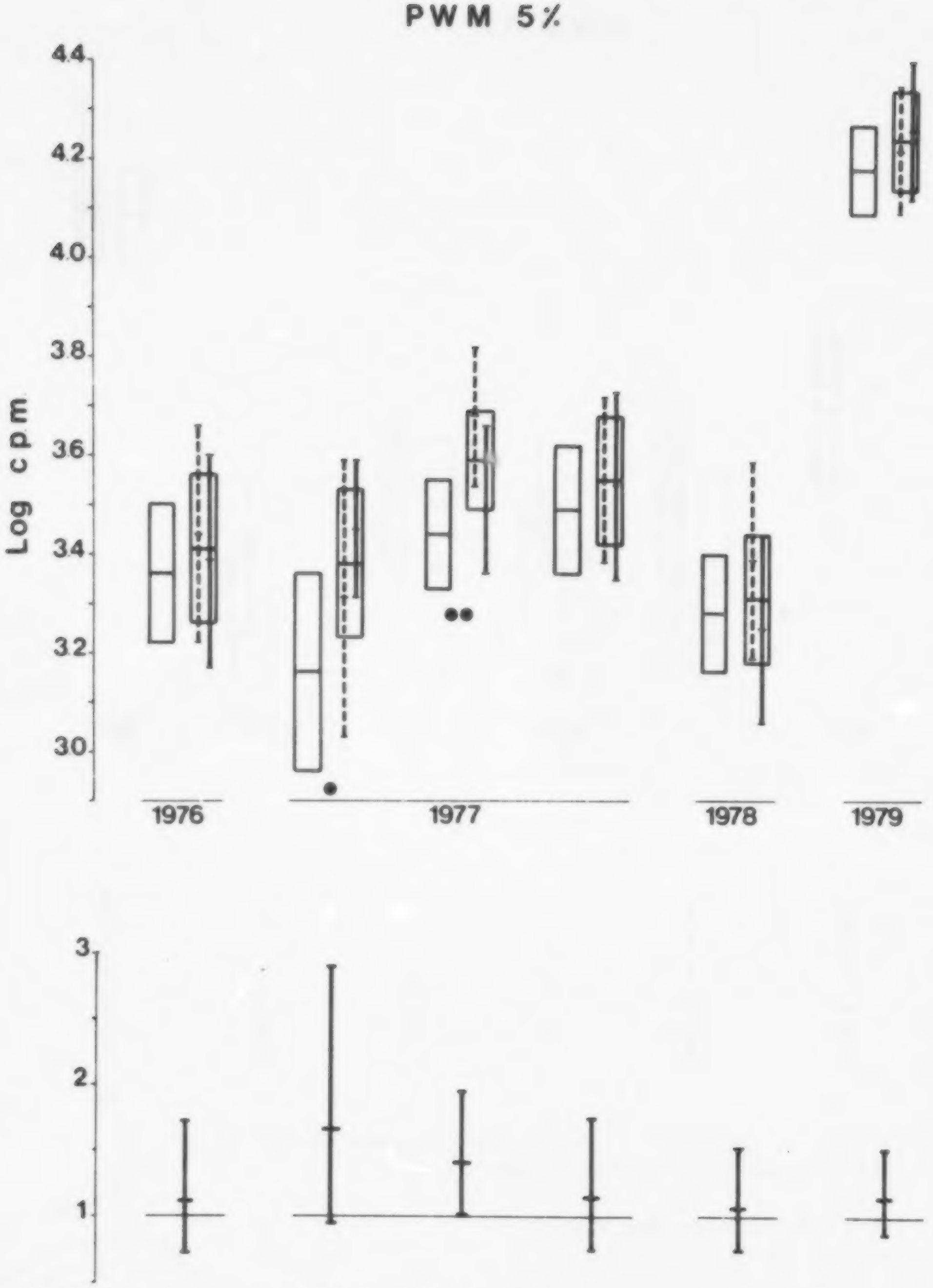


FIGURE 7 - Group of children. Lymphocyte response to PWM 5% (performed at Ospedale di Desio).

For the legend, see Figure 1.

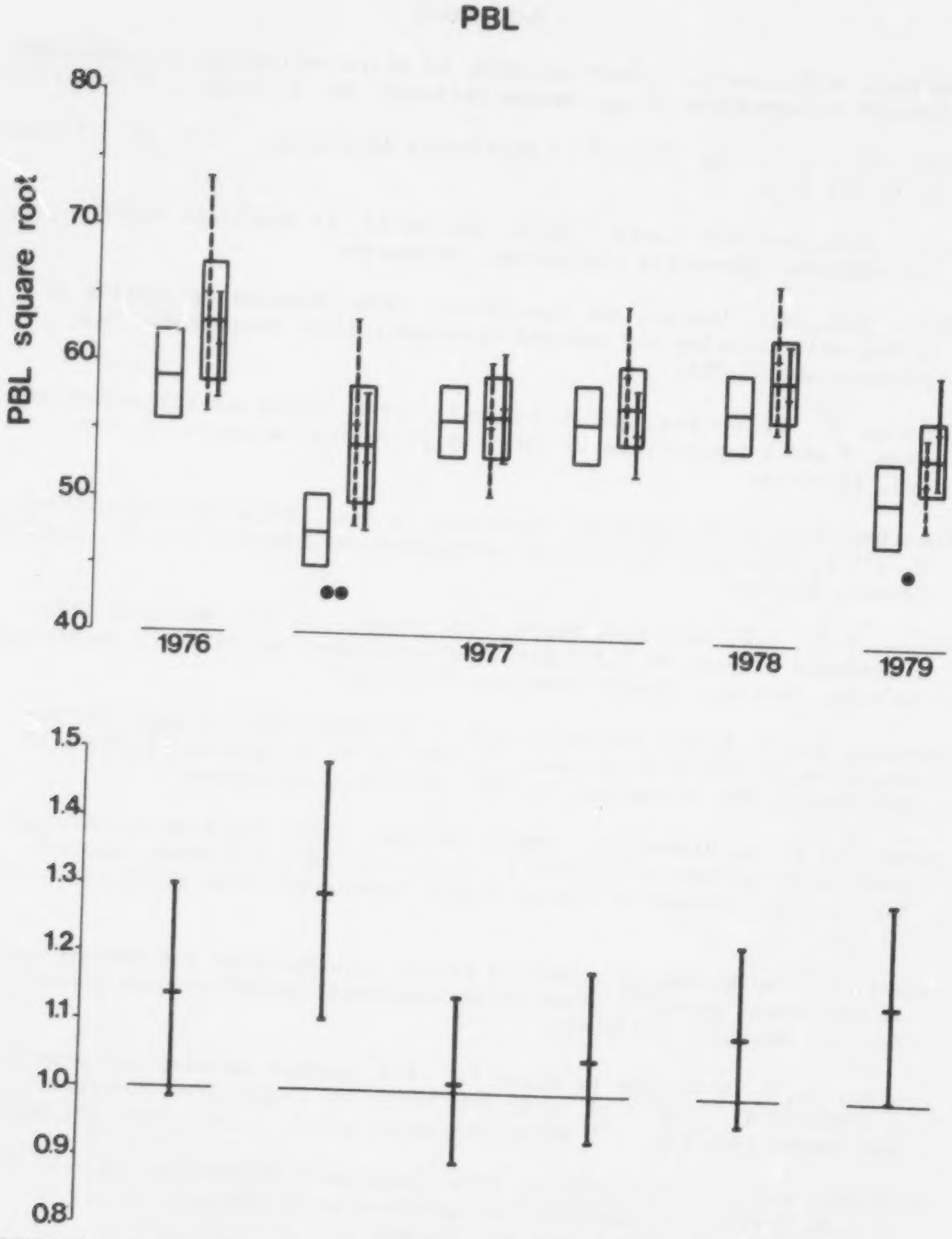


FIGURE 8 - Group of children. Number of peripheral blood lymphocytes. For the legend, see Figure 1.

REFERENCES

Anderson, R.E., and I. Lefkovits. 1979. In vitro evaluation of radiation-induced augmentation of the immune response. Am. J. Pathol. 97:456-472.

Bliss, C.I. 1970. Pp. 172-178 in Statistics in Biology. Vol. II. McGraw Hill, New York.

Dacie, J.V., and S.M. Lewis. 1975. Pp. 46-51, in Practical Haematology, 5th edition. Churchill Livingston, Edinburgh.

Faith, R.E., M.I. Luster, and J.A. Moore, 1978. Chemical separation of helper cell function and delayed hypersensitivity responses. Cell Immunol. 40:275-284.

Greaves, M., G. Janossy, and M. Doenhoff. 1974. Selective triggering of human T and B lymphocytes in vitro by polyclonal mitogens. J. Exp. Med. 140:1-18.

Guenther, W.C. 1977. Sampling inspection in statistical quality control. P. 155 in Griffin's Statistical Monographs and Courses, No. 37. Griffin, London, England.

Gupta, B.N., J.G. Vos, J.A. Moore, J.G. Zinkl, and B.C. Bullock. 1973. Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspect. 5:125-140.

Hadding, U., D. Bitter-Suermann, and F. Melchert. 1967. A tool for the detection of C'6 deficiencies. Pp.319-321 in H. Peeters ed. Vol. 17. Protides of the Biological Fluids. Elsevier, Amsterdam.

Helgesen, A., H. Hirschberg, and E. Thorsby. 1973. Modified micro-mixed lymphocyte culture technique. P. 75 in J.G. Ray, D.B. Hare, and D.E. Kayhoe, eds. Manual of Tissue Typing Techniques. DHEW (NIH) publication 74-545.

Huber, C., and H. Wigzell. 1975. A simple rosette assay for demonstration of complement receptor sites using complement-coated zymosan beads. Eur. J. Immunol. 5:432-435.

Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. Part I, A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. J. Exp. Med. 136:207-215.

Lachmann, P.J. and M.J. Hobart. 1978. Complement technology. Chapter 5A in D.M. Weir, ed. Handbook of Experimental Immunology, 3d ed. Blackwell Scientific Publications, Oxford, England.

Mancini, G., A.O. Carbonara, and J.F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235-254.

Moore, J.A., and R.E. Faith. 1976. Immunologic response and factors affecting its assessment. Environ. Health Perspect. 18:125-131.

Nye, L., T.G. Merret, J. Landon, and R.J. White. 1975. A detailed investigation of circulating IgE levels in a normal population. Clin. Allergy 5:13-24.

Owen, D.B. 1962. Handbook of Statistical Tables. Addison-Wesley Publishing Co., Reading, Mass.

Pandian, M.R., and G.P. Talwar. 1971. Effect of growth hormone on the metabolism of thymus and on the immune response against sheep erythrocytes. J. Exp. Med. 134:1095-1113.

Rosso di San Secondo, V.E.M., S. Milani, C. Fortis, E. Marubini, and G. Sirchia. 1979. The variability of lymphocyte transformation tests. Transplant Proc. 11:1379-1380.

Scheffe, J. 1961. Pp. 66-83 in The Analysis of Variance. John Wiley & Sons, New York.

Snedecor, G.W., and W.G. Cochran. 1972. Statistical Methods. The Iowa State University Press, Ames, Iowa.

Thigpen, J.E., R.E. Faith, E.E. McConnell, and J.A. Moore. 1975. Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Infect. Immun. 12:1319-1324.

Vecchi, A. A. Mantovani, M. Sizoni, W. Luini, M. Cairo, and S. Garattini. 1980. Effect of acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral antibody production in mice.

Vos, J.G., and J.A. Moore. 1974. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int. Arch. Allergy Appl. Immunol. 47:777-794.

Vos, J.G., J.A. Moore, and J.G. Zinkl. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ. Health. Perspect. 5:149-162.

World Health Organization Scientific Group on Immunodeficiency. 1979. Immunodeficiency Clin. Immunol. Immunopath. 13:296-359.

DISCUSSION

DR. MILLER: What about the ascertainment of mortality in the population? Is mortality being studied?

DR. SIRCHIA: No, this cohort is very small. I believe that mortality changes will become apparent over a very long period. This is also true for morbidity.

DR. DARDANONI: Dr. Sirchia is speaking about mortality within the sample studied, a rather small sample of the exposed population. I think you are referring to the entire population. Mortality and hospital morbidity are going to be monitored for the entire population through an assessment of general health indicators, such as mortality by age and sex, mortality from specific causes, and morbidity severe enough to require hospitalization. Data have already been collected, but not yet analyzed. There is circumstantial evidence that general morbidity has not changed.

DR. MILLER: What of individual causes of morbidity? When people develop infections, are the infections more severe if the people were exposed or if they had chloracne or not?

DR. DARDANONI: I do not think there is any approach to evaluate that.

DR. MOORE: Is any consideration being given to testing some of the population with recall antigen response or something like it?

DR. SIRCHIA: It was scheduled, but it was impossible because people did not comply. They did not want skin tines.

DR. MOORE: Does Italy vaccinate for TB? Could you use that as a recall type?

DR. SIRCHIA: Yes.

DR. DARDANONI: Unfortunately, that has not been done.

Somatic Cell Mutations

Arthur D. Bloom[1]

Environmental chemicals may induce both chromosomal and specific locus mutations. Chromosomal mutations in mammalian somatic cells include the classic forms of chromatid and chromosome-type lesions, as well as the more recently described sister chromatid exchanges, shown by differential staining techniques. Since specific locus mutation rates cannot presently be studied *in vivo*, several loci (in either cultured fibroblasts or cultured lymphocytes) must be analyzed to identify *in vitro* mutagenesis with known or putative agents. The two-step cancer hypothesis suggests that some individuals are genetically at increased risk of cancer. Induced somatic mutation in these individuals is superimposed on an inherited genic or chromosomal abnormality. Among the most promising direct mutation assays is one that determines the frequency of cells deficient in HGPRT (hypoxanthine-guanine phosphoribosyltransferase) among a wild-type population. At this time, the determination of somatic mutation in populations exposed to chemical mutagens is based on studies of clastogenesis and sister chromatid exchange, rather than on specific locus assays.

Genetic alterations of somatic cells exposed to environmental mutagens *in vivo* are of two basic kinds: chromosomal and genic. Chromosomal mutations induced in mammalian somatic cells after such exposures include the classic forms of chromatid- and chromosome-type lesions, involving, respectively, one or both arms of the chromosomes, which may undergo simple breakage or more complex breakage and rearrangement (Bloom, 1972).

In recent years, through the use of differential staining techniques in which only one arm of the chromosome is stained, the proportion of sister chromatid exchanges (SCE's) representing crossovers

---

[1] Departments of Pediatrics and Human Genetics, College of Physicians and Surgeons, Columbia University, New York.

between the chromatids of the same chromosome has been determined. Numerous reports have shown these SCE's to correlate well with the induction of single gene mutations, particularly when exposure is to chemical (rather than to physical) mutagens (Perry and Wolff, 1974). A last chromosomal effect involves alteration of the mitotic apparatus and resulting segregational errors. This leads to somatic nondisjunction and secondary trisomies and monosomies.

At the single gene level, a small number of loci can be used to study forward and reverse mutation in cultured mammalian cells. The principle, of course, is that the cells must be cultured; in practical terms, the effects of environmental contaminants can be studied _in vitro_, but as yet there is no technique to sample somatic cells directly and to determine the mutation rate for a specific locus _in vivo_.

The most promising direct assays are those involving a search for individual cells with abnormal, induced, mutant hemoglobins among a normal or wild-type population of red blood cells. Other promising assays analyze the presence of hypoxanthine-guanine phosphoribosyltransferase (HGPRT), the enzyme that is deficient in the X-linked Lesch-Nyhan syndrome. In this latter system, Albertini at the University of Vermont is attempting to isolate HGPRT-deficient blood cells from HGPRT-positive cells. Both the hemoglobin and HGPRT assays for direct mutation in somatic cells are in early developmental stages, and patients exposed to radiation or chemotherapy (i.e., exposed to known mutagens) are being sought for the study.

In the meantime, the effects of chemical mutagens at specific loci are being studied in cultured cells. The loci most commonly used are (1) the *hpt* locus, which specifies HGPRT synthesis; (2) the *tk* locus, which specifies the synthesis of thymidine kinase; and (3) the *oua* locus, which specifies resistance or sensitivity to the cardiomimetic compound ouabain, a membrane function determined by the enzyme $Na^+K^+ATPase$. A few other loci are sometimes used in these *in vitro* studies, but these three have been examined most thoroughly.

Many biologic and clinical effects of compounds such as DBCP (1,2-dibromo-3-chloropropane, an agricultural nematocide and known carcinogen) and TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; a highly toxic contaminant formed during 2,4,5-trichlorophenol production) have been reported. However, the somatic cell mutagenicity of these and related compounds causes the most concern. As described in their paper in this volume, Pocchiari *et al*. have recently reviewed the health effects of the Seveso accident involving TCDD, in terms of chloracne, peripheral nervous system effects, the possible increased incidence of abortions, and the possible increase in chromosomal aberrations in persons with chloracne. The long range concern, however, is cancer, because of the relationship between somatic cell mutations and neoplastic disease.

The suggestion by Knudson that some cancer, specifically retinoblastoma, develops through a two-step mutational process has led to the more general notion that a significant proportion of human cancers may result from a person's genetic predisposition, with or without superimposed environmental insult (Knudson, 1979). What began as a mutational hypothesis for a specific tumor type can now, appropriately modified, be accepted as a theoretical framework for numerous types of neoplasms. Thus, the mutational origins of human cancers are becoming clarified.

Some individuals are born with a genic or chromosomal mutation that puts them, *in utero* and thereafter, at increased risk of neoplastic disease. The underlying mutation can be vertically transmitted by parents who themselves may be at risk. Or, the inherited genic or chromosomal mutation may have arisen *de novo* during gametogenesis. The genes involved may be autosomal recessive or dominant. The recessive disorders studied most extensively to date are Fanconi's anemia (FA), Bloom's syndrome (BS), ataxia telangiectasia (AT), and xeroderma pigmentosum (XP). Deficiencies in DNA repair have been demonstrated for all but BS. The most fully studied autosomal-dominant disorders are retinoblastoma and Wilms's tumor. In each of these, one (in the dominant disorders) or two (for homozygotes in the recessive disorders and one in the heterozygous state) mutant genes are present from conception.

Similarly, karyotypic abnormalities can exert a similar effect. A deletion of chromosome 13 can be associated with retinoblastoma, and a deletion involving chromosome 11 is found in some Wilms's tumor cases. In addition, a chromosomal translocation (t3:8), seemingly balanced, was observed in a family with numerous cases of renal carcinoma. Translocation carriers have an 87% risk of renal carcinoma by age 57 (Cohen *et al.*, 1979). Thus, some persons begin life with a genetic predisposition to cancer; perhaps 1-2% of the people in the U.S. population carry these genes.

Superimposed on this background is somatic mutation. Some somatic mutations are spontaneous--of unknown cause; others are induced by identified environmental mutagens, either physical (such as ionizing radiation) or chemical. Although it is likely that massive exposure of a population to a strong mutagen will lead to carcinogenicity in many persons with or without underlying genetic predispositions, lower level exposures (particularly to mutagens of intermediate strength) may well act selectively on genetically at-risk persons.

It is somewhat ironic that, despite an enhanced understanding of the relationship between genic mutation and cancer, little more can be done now to detect exposure to environmental mutagens (in terms of somatic mutation) than was possible 15 years ago when the peripheral blood lymphocytes of A-bomb-exposed persons were being studied in Japan. Then, as now, cytogenetic monitoring was perhaps one of the most sensitive measures of mutagenic exposure.

In suspected incidents of areawide contamination, however, the sampling of peripheral lymphoctyes for chromosomal mutation studies is indicated. The proportion of aneuploid cells can be determined from such studies as well as the proportion of cells with chromatid and chromosome breaks and rearrangements. Clearly, SCE frequencies after *in vivo* exposures must also be determined on these same blood cells. Early studies on SCE's were conducted in cultured cells exposed to varying doses of known mutagens. At the 1980 meeting of the Environmental Mutagen Society, Carrano *et al*. (1980) described data on SCE frequencies in petroleum refinery workers. Of 22 workers studied, 11 had SCE frequencies more than two standard errors above the mean for the "nonexposed" group. The investigators reviewed the personal health and work histories of the workers and concluded that occupational or other environmental exposure (not smoking, medication, and so on) was probably responsible for the increased SCE.

Although the issue of genetic screening in the workplace is still controversial, identifying genetically at-risk persons may be lifesaving. Judiciously done, such examinations are a serious exercise in preventive medicine. For example, AT patients are known to be peculiarly susceptible to the effects of X-rays and gamma rays. Cells from FA patients are particularly susceptible to the effects of DNA crosslinking compounds. And yet, of the chromosome breakage syndromes, only XP cells have thus far been found found to be hypermutable _in vitro_. BS patients, however, have the highest spontaneous SCE frequencies and a marked response in terms of SCE increases when their cells are exposed to mutagens.

Thus, when dealing with exposures of a large population, it must be recognized that a subset (1-2%) of the population is more at risk than the rest. Specific individuals may not be identifiable by chromosome breakage and SCE studies after exposure. However, it may soon be possible to identify these persons generally with _in vitro_ tests that exert stress on cells by exposing them to chemical mutagens (as illustrated by the effect of diepoxybutane on FA cells) (Auerbach and Wolman, 1976).

In the next year or two, the Albertini HGPRT test should become helpful in estimating _in vivo_ somatic mutation rates. This test will use lymphocytes, incubated briefly in the selective medium 6-thioguanine (6TG), to isolate 6TG-resistant cells, which are hpt$^-$, forward mutants. The frequency of these cells in normal subjects is proving to be $1/10^4-10^5$ cells, a somewhat higher background rate than desired, perhaps, but not unmanageable.

Although this method will be valuable, it measures effects only at one locus, and might not provide reliable estimates of mutability at other loci. Ideally, multiple loci should be studied, and different kinds of genic products specified. The specific genes involved should be analyzed by restriction enzyme analysis. These techniques are not likely to be feasible in the near future because restriction enzyme technology currently requires both a large amount of DNA and specific mRNA probes (as is required for hemoglobin).

In summary, cytogenetic technology is still the best available for assessing genetic changes in somatic cells of exposed persons. In terms of specific locus mutation in somatic cells, known or putative mutagens can only be studied *in vitro*.

REFERENCES

Auerbach, A. D., and S. R. Wolman. 1976. Susceptibility of Fanconi's Anemia fibroblasts to chromosome damage by carcinogens. Nature 261:494-496.

Bloom, A. D. 1972. Induced chromosomal aberrations in man. Pp. 99-172 in H. Harris and H. Hirschhorn eds. Advances in Human Genetics, Vol. 3. Plenum Press, New York.

Carrano, A. V., J. L. Minkler, D. G. Stetka, and D. H. Moore, II. 1980. Variation in the baseline sister chromatid exchange frequency in human lymphocytes. Environ. Mutagen 2:325-337.

Cohen, A. J., F. P. Li, S. Berg, D. J. Marchetto, S. Tsai, S. C. Jacobs and R. S. Brown. 1979. Hereditary renal-cell carcinoma associated with a chromosomal translocation. N. Engl. J. Med. 301:592-595.

Knudson, A. G. 1979. Persons at high risk of cancer. (Editorial) N. Engl. J. Med. 301:606-607.

Perry, P., and S. Wolff. 1974. New Giemsa method for the differential staining of sister chromatids. Nature 251:156-158.

DISCUSSION

DR. MILLER: What studies would you recommend to examine body fluids or chromosomes of persons exposed to hazardous wastes to determine the biologic effects of a chemical mixture?

DR. BLOOM: We have to distinguish between biologic and clinical consequences. One major approach is to test for mutagenicity, because the correlation between mutagenicity and carcinogenicity is very high. So the approach is to examine body fluids, mixtures of chemicals (as from Love Canal), or individual chemicals in multiple tests across the prokaryotic and eukaryotic systems. For example, urine concentrates can be studied in the Ames assay; suspected carcinogens in certain fungi or yeast systems; and mammalian cell assays--mouse cell and human cell--can be helpful.

Estimates of mutation frequencies _in vitro_, how they apply _in vivo_, of course, remain to be determined. Theoretically, it is possible to determine whether or not individuals are exposed through the use of chromosomal assays and SCE frequencies in short-term cultured cells. Such evidence is, in a sense, all indirect. Most of the testing has to be performed _in vitro_. But some of it can be done directly, using these approaches. Somatic cell mutation studies are very limited.

DR. MILLER: Suppose you test 50 people and find that the Ames test is positive in 25 of the exposed group and in none of the control population. What does this mean?

DR. BLOOM: Well, the Ames test simply measures mutagenicity in an isolated strain of Salmonella. It won't be a direct statement of mutagenicity in vivo. A positive Ames assay says only that the compounds in the body fluids are, in fact, mutagenic.

Are the concentrations in body fluids in vivo the same as the concentrations producing mutation in vitro? There are usually enormous differences that make it difficult to extrapolate results from the in vitro test to the in vivo situation. Mutagenicity in the Ames test or in some other test systems usually occurs at dosage levels considerably higher than those found in vivo.

DR. MILLER: Can you identify the agent in urine that causes the Ames test to yield positive results?

DR. BLOOM: Yes, many chemical separation techniques can be used.

DR. DELLA PORTA: Do you have any good examples of the correlation of positive results for the Ames assay to an in vivo study of the same individual?

DR. BLOOM: Not for in vivo studies. Few studies have been systematic enough to definitely show that. Studies only show that a specimen is mutagenic in vitro.

DR. DELLA PORTA: Is there no example of the correlation based on results from the same individual?

DR. BLOOM: No.

DR. DARDANONI: Every person has some chromosomal aberrations of lymphocytes. Is it possible to choose a value that can discriminate between most of the pathologic and normal aberrations?

DR. BLOOM: Each laboratory has to standardize its own methods and determine the normal frequency of aberrations for individuals in that laboratory, particularly for persons of different ages. There is a lot of interlaboratory variation; no absolute value can be given for the frequency of chromosomal aberrations. Values should be described as either abnormal or normal for a specific laboratory.

DR. DARDANONI: If a test is designed to identify susceptible people, nonexposed people must be used as controls.

DR. BLOOM: Yes, that's true. The ideal is for an individual to be his or her own control (before or after exposure), but that doesn't usually happen.

DR. DELLA PORTA: Is the background noise higher when you look for chromosomal aberrations or for increased SCE's?

DR. BLOOM: Usually there is more variation in chromosomal aberration frequencies than there is in SCE frequencies. Again, each laboratory has its own baseline, and the variation around it tends to be much less than the discrepancy would be for a group of laboratories. The variation is much less around SCE's, perhaps because SCE's occur less frequently.

DR. NELSON: Tests on urine by an array of mutants, forward or reverse, are essentially surrogates for chemical analysis, which have a much greater sensitivity. Combining these tests with chemical separations and, perhaps, more selective tests might be of great value in analyzing exposure more sensitively. Screening body fluids for mutagenicity only examines exposure.

In some instances, the substances sought are transient, furtive, or labile. The exposure to be measured can have occurred yesterday, a few weeks ago, or even longer ago than that. Suppose that the exposed population at Love Canal had been removed from the area and kept in pristine, clean conditions. What approaches would you recommend for an historic review of genetic injury?

DR. BLOOM: If you are looking for genetic injury, the only persisting effects you are likely to see will be cytogenetic damage. A great deal of the damage induced by low-level exposure is ultimately repaired. The problem is to find a stable end point if you want to go back in time. That end point clearly has to be in the lymphocytes, which by chance are long-lived cells. Lymphocytes can be examined for chromosome breakage for a long time after exposure. Among specific genetic tests available now, an analysis of chromosome breakage is probably the only one that can be used.

DR. NELSON: What specifically needs to be done now to detect persistent genetic injury in somatic cells, that is, to identify chromosomal abnormalities, not SCE's?

DR. BLOOM: If the cell has undergone more than two replications after exposure, SCE's will not be detectable. It is now becoming more clearly established that short-term, high-dose _in vivo_ exposure to mutagens does induce detectable SCE's. Demonstration of an increase in SCE with longer term, low-dose, recurrent exposure is very likely, but has not yet been shown. SCE will probably not be visible or detectable many generations after high-dose exposure.

Cell-survival curves are determined in an interesting way. If the different types of chromosomal aberrations are classified according to involvement of only one arm of a chromosome, or of both arms (which produces rearrangements such as dicentric chromosomes) then different kinetics are involved. The frequency of chromosomal aberrations differs by type. The simpler kinds of chromosomal damage are more easily repairable. More complex damage, for example, dicentric chromosomes, tricentric chromosomes, rings, and translocations, tends to persist, particularly if the cell is not undergoing many cell divisions. Most lymphocytes in the peripheral blood are in G zero--they are quiescent.

The phenomenon of amplification can be added to the baseline level of induced aberrations. Under certain conditions, subpopulations of the lymphocytes will be triggered to divide, for example, by specific antigenic stimulation. In this way, chromosomally abnormal cells can be induced to form clones. A single cell that has a stable translocation, for example, can be antigenically stimulated, by a variety of means, to proliferate in vivo. Suddenly there may be not just a single copy of that cell in a million lymphocytes but several thousand copies. Is that a mutant, malignant clone? Some evidence indicates that such clone formation may well be related to the development of specific kinds of cancers that arise from single cells.

The biology, then, involves not only repair of damage to DNA with a resultant decrease in the frequency of chromosomal aberrations, but also an increase in the frequency of mutant clones within an exposed population of cells.

In vivo clone formation has not really been demonstrated with chemical carcinogens or mutagens. The formation has been shown with ionizing radiation. Whether it occurs after exposure to chemical carcinogens or not is unclear. Chemicals tend to be less efficient than ionizing radiation in inducing chromosomal abnormalities. On the other hand, SCE frequencies tend to be much higher with chemical carcinogens than with physical mutagens. There are differences and to some extent they are complementary.

DR. NELSON: Is antigenic stimulation convertible into a provocative test that would be safe and useful?

DR. BLOOM: That is the purpose of looking at the cells *in vitro* but it may not be a reasonable thing to do *in vivo*.

DR. DELLA PORTA: Is there any example of an exposure followed by chromosomal aberrations and cancer?

DR. BLOOM: There are many types of compounds, benzopyrene or benzene, for example, that can produce chromosomal damage in cell systems and *in vivo*; these compounds are associated with specific kinds of neoplasia. The Hiroshima-Nagasaki radiation studies are perhaps the classic example of that.

DR. DELLA PORTA: Vigliani's group described a relationship between chromosomal aberrations and leukemia in workers exposed to benzene. But has the exposure of a given population to a specific chemical been studied enough to define the cytogenetic abnormalities?

DR. MILLER: Two studies, of benzene and radiation, have been performed but we don't know that the chromosomal aberration leads to the leukemia.

DR. DE CARLI: What is the possibility of using unscheduled DNA synthesis to evaluate genetic injury in epidemiologic studies?

DR. BLOOM: It is probably a reasonable approach to try to identify individuals at risk in terms of their having deficiencies in DNA repair. This may not work in a population already exposed. The technique really requires rather short-term followup of cellular response to high-dose exposure. The problem is that screening for unscheduled DNA synthesis is a very nonspecific kind of test; it does not define the nature of the repair problem. An abnormal result relates to a general phenomenon (deficient repair); it reveals nothing about the mechanism. Probably many mechanisms are involved, particularly in mammalian cells.

DR. DELLA PORTA: What do you think is the mechanism underlying SCE's? Repair?

DR. BLOOM: The test uses bromodeoxyuridine to substitute uridine for thymidine. When up to two cell divisions occur, thymidine is replaced by uridine in one chromatid of a chromosome in a fair proportion of cells. The presence of uridine quenches the stain in the two chromatids. The mechanism is not clear. Presumably we are simply looking at a manifestation of somatic crossover. The results suggest that there are break points in the nucleotide sequence. There are at least two in most regional exchanges. Undoubtedly, specific target sites exist on the nucleotide sequence where many of these chemicals will act. Exactly where the breakpoints are has not been established.

The exchange that occurs was not detectable until these techniques became available. Compounds that are effective with regard to specific locus mutation are probably the same. These are the compounds that can induce increases in SCE frequencies. So SCE works in much the same way as point mutation does, and, therefore, is a very reasonable indicator of point mutagenicity.

DR. WEINBERG: Given that these test systems can be used for exposed populations at chemical dumpsites, and given that there is a large amount of variability from laboratory to laboratory, do you see the need for more careful planning of studies of several dumpsites? More specifically, should a central laboratory be designated to perform all the testing so that the results can be better compared?

DR. BLOOM: Yes. The Environmental Mutagen Society is considering the possibility of licensing laboratories for specific kinds of studies with chemical mutagens. These laboratories, each with expertise in a small number of bioassay systems, could probably be scattered around the world. Otherwise, many laboratories, particularly commerical ones, try to perform too many of these tests. Many firms will perform multiple kinds of assays, at times with minimal and superficial knowledge, even within the genetic test system, not to mention other kinds of test systems. They may be competent in cytogenetics, but not necessarily in specific locus mutation tests or in the Ames assay. Or they may do the Ames well, and know nothing about cytogenetics.

This problem surfaced in Harrisburg, for example, when the Three Mile Island accident occurred. Some of the tests obviously should have been performed right away. There was great discussion about which laboratory to use. Was the closest laboratory the best one? Was it even a reasonable laboratory for determining SCE's or chromosomal aberrations?

DR. MILLER: As I understand what you have said, if you had been called in August 1978 for the Love Canal incident, you would have recommended (1) SCE studies, (2) other cytogenetic studies, and (3) possible examination of urine in short-term assays. Is there anything else?

DR. BLOOM: The systematic examination of certain individuals such as those who were pregnant when exposed. A major somatic manifestation of many chemical carcinogens is teratogenicity. Thus, consideration should be given to examination of pregnant women and of their fetuses, perhaps using amniocentesis, and certainly to follow up of the newborn infants.

Various observations should be made, but the general medical approach really involves setting up cohorts and following the individuals for morbidity and mortality to watch for disorders that are likely to develop. The kinds of studies made in Seveso were appropriate.

DR. MILLER: In an exposed area, if a person has surgery, should a sample of skin fibroblasts be obtained for storage?

DR. BLOOM: Not really.

DR. MILLER: Suppose a woman miscarries; should a special study be made of the fetus?

DR. BLOOM: Absolutely.

DR. MILLER: Did Dr. Rehder suggest using the amnion if the fetus is macerated?

DR. REHDER: The fetus is mostly autolytic. Placental tissue will yield mostly maternal cells; this is a risk. You can exclude this possibility by studying the amniotic membrane.

DR. BLOOM: Certainly it is possible to culture the fetal cells.

DR. REHDER: Yes. It is certainly better to study fetal cells, if the fetus is not macerated.

DR. MILLER: What tissues or body fluids could be saved and by what methods? For instance, if the fetus is put in formalin, cytogenetic studies are not possible. And how should urine samples be handled? Should they be lyophilized or somehow reduced in volume? How can lymphocytes be preserved for future cytogenetic study?

DR. BLOOM: It is difficult. If you can separate the lymphocytes and put them in medium, they can be studied for days after an exposure. But after several weeks, nothing much can be done.

DR. MILLER: Could lymphocytes be processed to a point for cytogenetic analysis and stopped short?

DR. BLOOM: Absolutely. For example, white cells can be separated from blood. The buffy coat could be put into tissue culture medium--that would be the ideal if it were available--and left standing for days. That would rescue the lymphocytes. Freeze them for later use.

DR. HEATH: The sequence of events of what you would do in the case of another Love Canal is logical, but you pointed out that in the case of Three Mile Island, the choices are contingent on dosage considerations.

DR. BLOOM: Yes.

DR. HEATH: At Three Mile Island there was a lot of talk about cytogenetic studies, but the decision was made not to proceed because the dosage was only a fraction of the background levels. The exposure certainly was there, but the dosage to more than a few people around the canal was, perhaps, not very large. Would you still base that judgment on size of dose? I don't know what the judgment should have been at Love Canal, or should be if a similar situation were to occur. But size of dose is important to the decision to perform an expensive battery of tests.

The question has to do with your reference to an important aspect of preventive medicine--screening the workplace (or other) population particularly after an exposure. You said 1-2% would be predisposed to adverse effects. Where did that figure come from?

DR. BLOOM: Michael Swift at the University of North Carolina studied ataxia telangiectasia (AT). He has begun to estimate the frequency within the population of heterozygotes for AT and Fanconi's anemia. His estimate in the AT study was that about 1% of the general population may be heterozygous for the AT gene.

DR. HEATH: Would you add on the frequencies of other high-risk heterozygotes?

DR. BLOOM: Yes.

DR. HEATH: Has any such screening of the population in Japan taken place, to follow at least that cohort in terms of increased risk?

DR. BLOOM: No. It has not been established that these individuals can be detected by screening the general population.

DR. IREY: The chromosomal aberrations you are picking up are in the cell line of the lymphocytes. What is going on in other cell lines of the same organism?

DR. BLOOM: The presumption is that the lymphocytic response is simply a manifestation of what is going on throughout the body. On the other hand, there are undoubtedly tissue differences in susceptibility. Because we can only study certain tissues, we really do not know what these differences are. The lymphocyte-chromosome response is very likely the same as it is in other systems. When we see chromosomal damage in the lymphocytes and thyroid cancer, it makes sense to say that they are related.

DR. GOLDBERG: Have studies been conducted in an industrial setting of SCE's, somatic mutations, chromosomal aberrations, and positive urine results in the Ames test to give an idea of the influence of smoking on an industrial population?

DR. BLOOM: The SCE studies in the industrial setting are just beginning. Carrano's is perhaps one of the first, and that has only been described in abstract form. Studies of chromosome breakage in workers have been most heavily applied to the radiation industry, in Dow's plutonium workers and a few selected groups of that sort. Genetic screening in the workplace has not been widely performed.

DR. GOLDBERG: What about the Ames test?

DR. BLOOM: The Ames assay has been applied to urine from people working in a variety of industrial settings and from a variety of people exposed to putative mutagens. Results of the assay in general correlate well when one has a known mutagen, or metabolite of a known mutagen, in the urine. But there have been only a few such studies of workers.

DR. MILLER: In the past, policymakers in oncology said, "Why study genetics? You can't fix genetics." It now seems to me that genetics is being used to study the environment.

DR. BLOOM: That's right. Basically we are talking about the response of an organism to an environmental agent. We cannot alter the genetic response, but when genetic measures are known to be very sensitive, they can be used to help identify deleterious agents. They are of value in protecting the population. We are not trying to alter the genotype; we are trying to use the responses of the chromosomes, specific loci, and so on, as measures of environmental risk.

If somatic cell damage leads to clinical disease such as cancer or birth defects, it is assumed then that the mutational effects are likely to be reproduced in the germ cells because the meiotic chromosomal material there is essentially the same as it is in somatic cells. The end points are difficult to define; ways of estimating vertically transmitted damage are not always available.

Somatic cell effects are reasonable measures to use to identify those compounds in the environment that are capable of producing both somatic and potentially genic effects, which will be reflected in later generations.

My concern is primarily with somatic effects. Unless a really massive exposure to a potent mutagen occurs, it is unlikely that environmentally induced, inherited abnormalities can be detected. If we could not detect them after the atomic bomb exposures in Japan, it is very unlikely that we will identify them anywhere else.

DR. MILLER: What about the people exposed to Kepone or DBCP? Should their children be studied by biochemical genetics?

DR. BLOOM: I think not. Unless the numbers are sufficiently large, it would not be worthwhile.

DR. TARDIFF: With respect to several classes of chemical mutagens and chemical carcinogens, advances have been made in the last several years in measuring the formation of adducts to nuclear material. Presumably an initiating step leads to an improper replication leading to other events. Please comment on the possibility of using that kind of analysis as an adjunct to measuring mutagenic activity of SCE.

DR. BLOOM: Such studies are really in an early stage. Undoubtedly some mutagens are going to work on cell membranes; others are going to work directly on the DNA. Some mutagenic compounds are going to affect cell replication. We are calling all of these substances mutagens, even though they may not all be mutagens. Defining the end point is very different from defining the mechanism by which a mutagen works.

When we talk about chromosomal aberrations or SCE, we are not really discussing the mechanism by which an event takes place. For instance, the range of mechanisms is enormous for nitrosamines versus alkylating agents. To generalize is not necessarily reasonable.

DR. TARDIFF: For those agents known to produce electrophiles and for which there is a substantial likelihood that there would be adduct formation with genetic material, wouldn't it be reasonable to study adduct formation under those circumstances? Of course, while adducts do not necessarily lead to an immediate expression of a genetic alteration, genetic alterations could occur at a substantially later time.

Can this mechanism be used to identify people whose risk may not be immediately observable, but who may themselves be at risk in the future, or, perhaps, whose progeny may be at risk from parental exposure?

DR. BLOOM: How would you identify them?

DR. TARDIFF: If one could identify people with adduct formations (as a result of exposures to certain materials that are metabolized to electrophiles), one might then be able to follow those people as unique study populations to find out if, in fact, their risk materializes and to what extent. Then perhaps one could use the information to predict effects for other, similarly exposed populations.

DR. BLOOM: I am not aware of any applications of that technology in large-scale human studies.

DR. TARDIFF: Might it be feasible to apply the technique to populations that have known exposures to agents that have this kind of mechanism?

DR. BLOOM: Yes, it's possible.

DR. MILLER: Dr. Rehder and Dr. Gropp have studied embryos from Seveso. What are your findings?

DR. REHDER: We studied 32 fetuses from pregnancies interrupted at the mothers' requests, because the women said they had been exposed. Analysis of where these women lived revealed that none of them had been heavily exposed. Those who were exposed were exposed for a very short time. Most of the women said they had been exposed while riding through the area in cars.

Abortions were performed by curettage, rather early in gestation, and most of the material was lost. Our publication lists the study samples, but mostly we had only one leg and a bit of placenta. We did not have whole fetues, so we could not say whether or not they were deformed. In the few cases where we had complete fetuses, we found no malformations except for lesions caused by the abortions. We also studied specimens from three spontaneous abortions; these did have anomalies. In one, there was a total infarction of the placenta, which would explain the abortion; another had atypical facies, a heart malformation, and some minor internal stigmata. This fetus probably had chromosomal aberrations and also Down's syndrome.

The third case had a molar degeneration of the placenta, which is not a true mole. It is a form of degeneration found frequently in chromosomal aberrations, with a closed amniotic sac, but without an embryo--like a blighted ovum. Chromosomal aberrations have been found in samples such as this, for example, by the Bovés in Paris. Of course, some alterations would be expected in the case of these spontaneous abortions--something to explain the abortion.

We did not see anything abnormal in the fetuses from the induced abortions. Our conclusion, thus, was that we did not find anything beyond the normal incidence of malformations.

One year later, we analyzed another 50 cases of spontaneous abortions and neonatal deaths located by Dr. Sancioni, and we analyzed all of the malformations she had found. Of these, 24% had major and minor malformations and cases of trisomy 13 and 18. Again, we compared our findings to those of another sample from outside the area and to published data on malformations, aberrations, and minor anomalies among newborns. The results from the 50 cases were below the ranges established by the comparison groups. Although the 24% rate had seemed high at the beginning of the study, it really was not above the norm. The malformations and anomalies were very heterogeneous; there was no specificity at all.

Cytogenetic Investigations of the Seveso Population Exposed to TCDD

L. De Carli, A. Mottura, F. Nuzzo, G. Zei, M.L. Tenchini, M. Fraccaro,
B. Nicoletti, G. Simoni, and P. Mocarelli[1]

The scoring of chromosomal aberrations in fibroblasts and lymphocytes from subjects exposed to toxic compounds is of limited value as an indicator of somatic genetic damage. Nonetheless, it is the only method available to evaluate immediate effects on genetic material. After the Seveso accident, the Institutes of General Biology and Genetics of the universities of Milan, Pavia, and Rome began a cooperative program of cytogenetic investigations of individuals from the TCDD-contaminated area. Chromosomal analysis was performed on 28 cases of induced abortions for a comparison with the frequencies of chromosomal aberrations in cultured cells from maternal and fetal tissues. A sample of 301 individuals from the Seveso population was also investigated. This study included 145 residents of Zone A near the ICMESA (Industrie Chimiche Meda Societá Anonima) farm; 69 nonresident ICMESA workers; and 87 control subjects.

The differences in the frequency of aberrant cells between exposed and control subjects were not generally significantly greater than those among individuals and among observations from different laboratories. However, in some cases, the frequency of mitotic samples with at least one aberrant cell was higher in the exposed groups than in controls. The results so far do not suggest a straightforward conclusion, but indicate the need to continue the cytogenetic monitoring of the Seveso population.

The detection of chromosomal aberrations in lymphocyte or fibroblast cultures from exposed individuals is virtually the only immediate and practical tool for evaluating genetic damage induced by a toxic substance accidentally released into the environment (Evans and O'Riordan, 1975). Genic mutations cannot be detected so easily. The possibility of recognizing them in cells from tissues of exposed individuals is confined to a few loci in the hemizygous state (Albertini and De Mars, 1973; Strauss and Albertini, 1977). Since lymphocytes and fibroblasts are almost ubiquitous cells, they may be regarded as targets that are representative of the organism, although the sensitivity of different tissues can vary greatly.

---

[1] Cytogenetics Collaborative Group, Regione Lombardia, Italy
Project coordinator: G. Morganti

The significance of chromosomal lesions in cells cultured in vitro for the pathology of an individual and progeny varies. An abnormally high frequency of chromosome breaks, when the capacity to repair DNA damage is normal, indicates that a chemical or physical agent has directly or indirectly interacted with the genetic material to bring about a change in its structure. However, different types of chromosomal aberrations have different genetic consequences, either at the somatic or germinal level. Completely or partially unstable aberrations (chromatid and chromosome breaks, rings, and dicentrics) are relevant mainly for cell survival and for the control of cell proliferation insofar as they can determine cellular lethality or can represent a critical step in the succession of events ending in neoplastic transformation (Bloom, 1972). Stable chromosome aberrations (translocations and inversions) may represent transmissible genetic damage, and therefore can indicate a risk to progeny. However, a sizable fraction of chromosome structural variation detectable in vitro may not have any pathologic significance because it does not reflect the condition in vivo. A variety of factors inherent to cell culture, including pH, quality of medium, and contamination by mycoplasma, induce chromosomal alterations (Keck and Emerit, 1979; Schneider et al., 1974). The presence of either chromatid or isochromatid and chromosomal aberrations is generally considered a criterion for discriminating preexisting chromosome damage from that produced in vitro, but it is not absolutely valid. Due to the interference of technical factors, the cell cultures from exposed persons must always be established in tandem with those of controls. Moreover, data on the incidence of chromosome lesions in cell samples cultured in different laboratories or at different times are hardly comparable.

Finally, scoring for chromosomal aberrations *in vitro* is informative only if a positive response, providing evidence of a deleterious change in the genetic material, is observed. A negative response is not sufficient to exclude the occurrence of such a change, even at a chromosomal level (Schinzel and Schmid, 1976).

Despite all these limitations, cytogenetic analysis of exposed subjects appears to be unavoidable, at least for those cases in which the mutagenicity of a chemical contaminant can be predicted on the basis of results of laboratory tests or from inferences drawn from the molecular structure of the compound. Such is the case with tetrachlorodibenzodioxin (TCDD), a weak mutagen both in prokaryotes and lower eukaryotes (Wasson *et al.*, 1978).

After the Seveso accident of July 10, 1976, the first chromosome analyses were performed at the request of hospitals. Analyses were performed on blood from eight children aged 2 to 10 years and from four pregnant women living in the contaminated area and admitted to the hospitals because of dermatologic lesions, presumably caused by TCDD exposure.

A second sample group included 28 women who chose abortion because of a presumed risk of teratogenesis. Of these, 21 were in the first trimester of pregnancy at the time of the accident; 7 had become pregnant immediately afterward. Preliminary data on these cases have already been reported (Tenchini et al., 1979). At that time, Italian law did not permit interruption of pregnancy. Therefore, a parallel sample could not be obtained for nonexposed, interrupted pregnancies. Such samples were studied later, after abortion was legalized in Italy for clinically normal cases, which could serve as suitable controls. Thus, samples from 16 pregnancies became available for cytogenetic study some 2 years later.

In a collaborative program sponsored by the administration of the Regione Lombardia, five Italian laboratories have undertaken a more extensive cytogenetic analysis of the Seveso population. During a 5-month period starting in October 1976, investigators sampled 330 persons, who were distributed into three exposure categories: acute--162 persons who lived near the ICMESA plant in Zone A; chronic--73 persons who worked at the ICMESA plant but did not live in Zone A; and controls--95 persons who lived in the area surrounding the contaminated zone and matched exposed persons by age and sex.

The types of chromosomal aberrations studied were (1) chromatid and isochromatid gaps and chromatid and chromosomal breaks and (2) chromatid and chromosomal rearrangements (chromatid interchanges, rings, dicentrics, and morphologically atypical chromosomes). Examples of these aberrations are illustrated in Figure 1. To evaluate the total amount of chromosome damage, the overall frequencies of chromosome lesions were established, including and excluding gaps, because the latter type of alterations is a poor indicator of cytogenetic risk. In the analysis of the large sample from the Seveso population, care was taken to ensure that the scorer did not know the classifications of the subjects under examination.

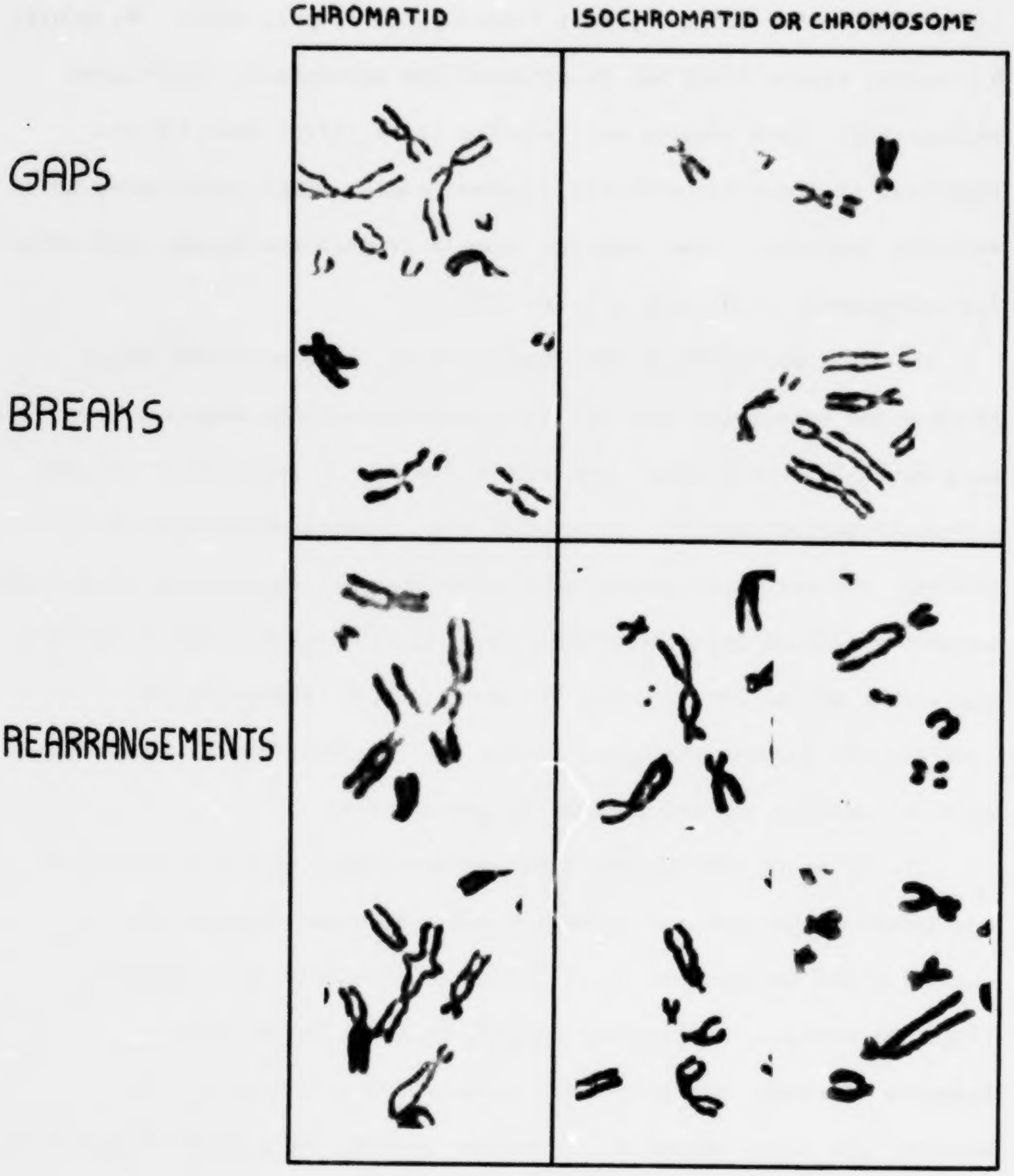

CHROMATID
ISOCHROMATID OR CHROMOSOME
GAPS
BREAKS
REARRANGEMENTS

FIGURE 1. Types of chromosome aberrations investigated at Seveso.

SAMPLE OF HOSPITAL PATIENTS

The frequencies of chromosomal aberrations in lymphocytes from 72-hour cultures of peripheral blood are shown in Table 1. Standard laboratory values, calculated on 10 adults examined during a comparable period are also provided for reference. The difference in the frequencies of chromosomal aberrations between the patients and the unmatched controls is not significant, except for the category of "aberrations including gaps" in pregnant women, which is at the limit of significance.

SAMPLE OF INTERRUPTED PREGNANCIES

For each pregnancy, fragment cultures were made from fetal tissues, placenta, and umbilical cord. Lymphocyte cultures were established from maternal blood drawn immediately before the abortion. Not all types of tissue were available for all cases. For the controls, particular care was taken to ensure that culture conditions, cytologic procedures, and scoring criteria were as identical as possible to those used for analyses of samples from exposed pregnancies.
The frequencies of chromosomal aberrations observed in cells from maternal and fetal tissues in exposed and nonexposed pregnancies are reported in Table 2. Data are recorded only for those specimens that showed a high growth rate within the first month of culture. The frequencies of chromosomal aberrations are consistently different between tissues within categories. Blood cultures have the lowest values and the placenta and umbilical cord cultures have values about twice as high. In the controls, fetal cells have aberrational frequencies comparable to those of the other solid tissues. In the exposed pregnancies, fetal cells have values almost twice as high.

TABLE 1

Frequencies of Chromosomal Aberrations in Blood Cultures of Hospital Patients

Subject	No. of Individuals	No. of Mitoses	% Aberrant Cells Including Gaps	 Excluding Gaps
Children	8	439	5.92 t^{a} = 1.4057 n.s.[b]	1.59 t = 0.4727 n.s.
Pregnant women	4	185	7.57 t = 2.4367 P = < 0.05	1.62 t = 0.4353 n.s.
Controls	10	553	3.98	1.99

---

[a] t = test of significance for comparison between two percentages (transformed values).

[b] n.s. = not significant ($P > 0.05$).

TABLE 2

Frequencies of Chromosomal Aberrations in Maternal and Fetal Tissues; Sample of Interrupted Pregnancies

Type of Tissue	Categories	No. of Pregnancies	No. of Mitoses	% aberrant cells ± s.d.[a]	
				Including Gaps	Excluding Gaps
Maternal blood	Control	11	1172	4.10 ± 1.13	2.22 ± 0.84
	Exposed	13	1403	3.35 ± 0.94	1.78 ± 0.69
				t = 0.93 n.s.	t = 0.73 n.s.
Placenta and umbilical cord	Control	11	1547	7.50 ± 1.31	4.91 ± 1.08
	Exposed	12	2001	8.40 ± 1.22	4.50 ± 0.91
				t = 0.99 n.s.	t = 0.56 n.s.
Fetal tissue	Control	12	1600	7.56 ± 1.30	4.44 ± 1.01
	Exposed	12	2050	15.17 ± 1.55	9.12 ± 1.25
				t = 7.24 P < 0.001	t = 5.68 P < 0.001

---

[a] s.d. = standard deviation

The differences among categories within tissues are not statistically significant for maternal blood, placenta, and umbilical cord. They are highly significant for fetal tissue.

SAMPLE OF SEVESO POPULATION AND ICMESA WORKERS

Four blood cultures were established for each individual, and preparations were made after 48 and 72 hours of incubation. To ensure homogeneity of cell culture conditions and cytologic procedures, this part of the work was concentrated in a single laboratory, the Institute of General Biology at the University of Milan. The coded slides were randomly distributed for a preliminary examination among the five laboratories participating in the program. The frequencies of the various types of aberrations have been determined on all the scorable preparations obtained from the 72-hour cultures. Although these preparations were less informative for determining preexistent chromosomal damage *in vivo*, they did permit analysis of a larger number of individuals than did the 48-hour preparations, which did not always show a sufficient yield of mitoses.

The number of cells analyzed per person in the first scoring ranged from 30 to 80. The cytogenetic analysis was performed on blood cultures and preparations were made at 48 and 72 hours. Complete data are available for 72-hour cultures. The pooled data from the different laboratories are shown in Table 3. Variance analysis was performed on the individual values, according to the criteria of exposure categories and scorers in different laboratories. The results are shown in Table 4. The differences in the frequencies of chromosomal aberrations, including gaps between categories, are at the limit of significance when compared with the differences among

TABLE 3

Frequency of Chromosomal Aberrations in Lymphocytes; Preliminary Cytogenic Analysis of Samples from the Seveso Population

Category	No. of Individuals	No. of Mitoses	% Aberrant Cells (Variance Interval)	
			Including Gaps	Excluding Gaps
Chronic exposure	69	3040	2.53 (0.00 - 12.00)	0.92 (0.00 - 12.00)
Acute exposure	145	6470	2.49 (0.00 - 18.00)	0.99 (0.00 - 8.00)
Controls	87	3958	1.64 (0.00 - 8.90)	0.48 (0.00 - 4.00)

TABLE 4

Variance Analysis on Transformed Values (arc sin $\sqrt{p}$) of Frequencies of Chromosomal Aberrations in Lymphocytes; Preliminary Cytogenetic Analysis; Samples from the Seveso population

Sources of Variation	S.S.[a]	d.f.[b]	Variance	F[c]	Statistical Significance
Including gaps					
Total	9880.3552	247			
Exposures	252.7708	2	126.3854	3.4499	< 0.05
Laboratories within exposure	981.8648	9	109.0961	2.9780	< 0.005
Individuals within laboratory, within exposure	8645.7196	236	36.6344		
Excluding gaps					
Total	4935.8563	247			
Exposures	102.2117	2	51.1059	2.5987	< 0.05
Laboratories within exposure	192.4735	9	21.3859	1.0875	< 0.05
Individuals within laboratory, within exposure	4641.1711	236	19.6660		

---

[a] S.S. = sum of squares.

[b] d.f. = degrees of freedom.

[c] F = variance ratio.

individuals within categories. However, the differences among scorers are highly significant. When gaps are excluded, these differences are no longer significant.

The results of the statistical analysis of these preliminary data prompted the extension of the analysis to a larger number of mitoses on selected samples from the three exposure classes. The samples had to be comparable in size and characterized by a high mitotic yield on both the 48- and 72-hour cultures. Each group contained samples from 45 persons and was subdivided into subgroups of 15 each. The subgroup samples were distributed among three laboratories according to the scheme shown in Table 5. Thus, preparations for each person were scored by two different laboratories.

The frequencies of chromosomal aberrations, both including and excluding gaps, and the number of aberrations per cell for the control, for both the acute and the chronic exposure categories, are reported in Tables 6, 7, and 8. Data from 48- and 72-hour cultures are recorded for each subgroup and for each of the two scorers. The variability in observations among the different laboratories is notable. Table 9 shows the average percentage of aberrant cells for the sets of 30 individuals scored in each category by each laboratory. Whereas two of the laboratories show consistently higher scorers for both chronic and acute exposure categories (as compared to the controls), the third shows almost equal values for the acute exposure category and the controls, but lower values for the chronic category.

TABLE 5

Experimental Design: Balanced Incomplete Blocks

	Exposures								
Laboratory[a]	Acute			Chronic			Controls		
PV^G	B_1	B_2		B_4	B_5		B_7	B_8	
R^B	B1		B_3	B_4		B_6	B_7		B_9
PV^B		B_2	B_3		B_5	B_6		B_8	B_9

---

[a] PV^G = Istituto di Genetica, Universitá di Pavia

R^B = Istituto di Biologia Generale, Universitá di Roma

PV^B = Istituto di Biologia Generale e Genetica Medica, Universitá di Pavia

TABLE 6

Frequencies of Chromosomal Aberrations for the Control Category, Analyzed in a Block Design; Sample of Seveso Population

Blocks	No. of Individuals	Laboratories	No. Scored per Block	No. of Mitoses	% Aberrant Cells Including Gaps	% Aberrant Cells Excluding Gaps	No. Aberrations/Cell Including Gaps	No. Aberrations/Cell Excluding Gaps
48 hours								
B_7	15	R^B	15	962	1.247	0.415	1.083	1.000
		PV^G	15	1144	0.786	0.174	1.000	1.000
B_8	15	PV^G	15	1482	0.877	0.269	1.000	1.000
		PV^B	15	1197	0.334	0.167	1.000	1.000
B_9	15	PV^B	9	483	2.070	1.035	1.200	1.200
		R^B	10	659	1.669	1.062	1.090	1.000
72 hours								
B_7	15	R^B	11	1027	1.168	0.681	1.250	1.000
		PV^G	14	1276	0.628	0.078	1.000	1.000
B_8	15	PV^G	15	1536	0.520	0.000	1.000	0.000
		PV^B	15	1475	1.423	0.813	1.142	1.000
B_9	15	PV^B	10	783	1.149	0.255	1.000	1.000
		RB	6	558	0.716	0.179	1.000	1.000

TABLE 7

Frequencies of Chromosomal Aberrations for the Acute Exposure Category Analyzed in a Block Design; Sample of Seveso Population

Blocks	No. of Individuals	Laboratory	No. Scored per Block	No. of Mitoses	% Aberrant Cells		No. Aberrations/Cell	
					Including Gaps	Excluding Gaps	Including Gaps	Excluding Gaps
48 hours								
B_1	15	PV^G	12	1173	0.767	0.255	1.000	1.000
		R^B	14	1250	1.200	0.320	1.133	1.000
B_2	15	PV^G	15	1540	1.363	0.324	1.000	1.000
		PV^B	15	1059	3.871	1.605	1.170	1.235
B_3	15	R^B	15	1415	1.625	0.565	1.000	1.000
		PV^B	15	1400	3.000	0.785	1.166	1.272
72 hours								
B_1	15	PV^G	12	1193	1.424	0.335	1.058	1.000
		R^B	14	1176	1.275	0.595	1.200	1.142
B_2	15	PV^G	15	1510	2.317	0.993	1.085	1.000
		PV^B	15	1650	3.575	1.939	1.050	1.062
B_3	15	R^B	14	1254	0.956	0.558	1.250	1.000
		PVB	15	1490	2.281	0.939	1.147	1.071

TABLE 8

Frequencies of Chromosomal Aberrations for the Chronic Exposure Category, Analyzed in a Block Design: Sample of Seveso Population

Blocks	No. of Individuals	Laboratories	No. Scored per Block	No. of Mitoses	% Aberrant Cells		No. Aberrations/Cell	
					Including Gaps	Excluding Gaps	Including Gaps	Excluding Gaps
48 hours								
B_4	15	PV^G	10	868	1.036	0.345	1.000	1.000
		R^B	14	1385	0.433	0.072	1.000	1.000
B_5	15	PV^G	11	592	3.209	1.520	1.000	1.000
		PV^B	11	489	2.658	0.817	1.000	1.000
B_6	15	R^B	13	1250	1.120	0.480	1.000	1.000
		PV^B	13	1142	1.488	0.437	1.176	1.000
72 hours								
B_4	15	PV^G	9	801	1.872	0.624	1.000	1.000
		R^B	14	1354	0.516	0.221	1.000	1.000
B_5	15	PV^G	9	844	1.895	0.947	1.125	1.125
		PV^B	13	1083	2.123	1.015	1.130	1.000
B_6	15	R^B	13	1300	0.307	0.076	1.250	2.000
		PVB	13	1170	1.965	0.598	1.217	1.428

TABLE 9

Frequencies of Chromosomal Aberrations for Each Exposure Category and Each Laboratory

Laboratories	Acute Exposure, % Aberrant Cells (No. of Mitoses)			Chronic Exposure, % Aberrant Cells (No. of Mitoses)			Controls, % Aberrant Cells (No. of Mitoses)		
	Including Gaps		Excluding Gaps	Including Gaps		Excluding Gaps	Including Gaps		Excluding Gaps
48 h									
PV^G	1.105	(2713)	0.294	1.917	(1460)	0.821	0.837	(2626)	0.228
R^B	1.425	(2665)	0.450	0.759	(2635)	0.265	1.418	(1621)	0.678
PV^B	3.375	(2459)	1.138	1.839	(1631)	0.551	0.833	(1680)	0.416
72 h									
PV^G	1.923	(2703)	0.702	1.884	(1645)	0.790	0.568	(2812)	0.035
R^B	1.111	(2430)	0.576	0.414	(2654)	0.150	1.009	(1585)	0.504
PV^B	2.961	(3140)	1.464	2.041	(2253)	0.798	1.328	(2258)	0.620

For the variance analysis, individual values were transformed by the $\sin^{-1}\sqrt{p}$ transformation. Only mitotic samples for which exact 100 counts were available were included in the analysis. The analysis was based on the following model.

$$Y_{ijq} = \mu + \P_i + \beta_{ij} + \tau_q + \epsilon_{ijq}$$

where are represented the effects of

μ = mean
$\P$ = exposure
β = incomplete block
τ = laboratory
ε = intrablock residual or error

Analysis of Variance

Sources of Variation:	d.f.
Exposures:	(c-1)
Laboratories (unadjusted):	(t-1)
Block within exposures (adjusted):	(b-c)
Intrablock error:	(tr-t-b+1)

where t = 3 = no. of laboratories, r = 6 = no. of replications, and b = 9 = no. of blocks. This is repeated for c = 3 = no. of exposures.

The results are shown in Tables 10 and 11. No significant differences were observed between exposure categories for any of the four combinations (48 and 72 hours, with aberrations inclusive or exclusive of gaps). Significant differences were observed between laboratories in the frequencies of aberrations at 72 hours of culture. The differences at 48 hours are insignificant.

An alternative criterion for analyzing differences in the induced chromosomal damage among exposure categories would be to use the proportion of individuals with at least one aberrant cell in either one or the other of the two scoring replicates. Such an analysis seemed particularly relevant because of the pronounced variability among subjects in the frequencies of chromosomal aberrations and also because the exposure conditions were most likely not homogenous. The values calculated in this way are shown in Table 12. A significant chi-square value was recorded for aberrations including gaps at 48 hours of culture because of the lower proportion of individuals with aberrations in the chronic exposure category.

Conclusions

From the analysis of the cytogenetic findings in the samples from TCDD-exposed persons, the following conclusions can be drawn. Owing to the limited size of the hospital patient sample and to the lack of controls from appropriately matched individuals, the results of the cytogenetic analysis are not very informative. Nevertheless, the average frequencies of chromosomal aberrations for the two groups

TABLE 10

F-Ratios in Analysis of Variance for the Comparison Between Exposures, Following the Balanced Incomplete Block Design

Aberrations	Culture Period, hours	s^2 Between Exposures/ s^2 Error = F (2,13 d.f.)[a]	Statistical Significance
Including gaps	48	$\frac{4.3742}{5.4036} = 0.8095$	n.s.
	72	$\frac{4.2779}{1.9854} = 2.1547$	n.s.
Excluding gaps	48	$\frac{2.4329}{5.2974} = 0.4593$	n.s.
	72	$\frac{5.7274}{2.5826} = 2.2176$	n.s.

---

[a] F (2, 13 d.f.) = variance ratio for 2 and 13 degrees of freedom.

TABLE 11

F-Ratios in Analysis of Variance for the Comparison Between Laboratories, Following the Balanced Incomplete Block Design

Aberrations	Culture Period, hours	s^2 Between Laboratories/ s^2 Error = F (2,7 d.f.)[a]	Statistical Significance
Including gaps	48	$\frac{7.7967}{2.3325} = 3.3426$	n.s.
	72	$\frac{17.1429}{1.1107} = 15.4343$	$P<0.01$
Excluding gaps	48	$\frac{1.2686}{3.2146} = 0.3946$	n.s.
	72	$\frac{9.4400}{1.7693} = 5.3355$	$P<0.05$

---

[a] F (2, 7 d.f.) = variance ratio for 2 and 7 degrees of freedom

TABLE 12

Overall Frequencies of Individuals with Chromosomal Aberrations

Exposure Categories	No. of Individuals	% of Individuals with Chromosomal Aberrations	
		Including Gaps	Excluding Gaps
48 hours			
Acute	29	96.55	62.07
Chronic	19	73.68	42.11
Controls	13	92.31	53.85
		$X^2 = 6.1418$ $P < 0.05$	$X^2 = 1.8428$
72 hours			
Acute	31	93.55	80.65
Chronic	23	86.96	56.52
Controls	27	85.19	55.56
		$X^2 = 1.1434$ n.s.	$X^2 = 5.1437$ n.s.

of patients are not significantly different from the standard laboratory values for the relevant period.

The data regarding the differences between blood and other tissues obtained from control pregnancies are consistent with those obtained from exposed pregnancies 2 years earlier. Placental and umbilical cord tissues showed frequencies of chromosomal aberrations two times higher than those of maternal blood, a finding that correlated well with the ratio usually observed between values in cells from solid tissues and in lymphocytes (Simoni et al., 1979). A significant deviation from this trend was found only for fetal cells from exposed pregnancies. These showed a fourfold increase in chromosomal aberrations with respect to maternal blood. It is difficult to ascribe such a difference to factors inherent in the cell culture even if established at different periods, because the values are comparable in the two samples for all other tissues. Therefore, the possible effect of TCDD exposure on the mothers has to be considered. Under this assumption, a transplacental action of TCDD and a higher sensitivity of the fetal cells to the chemical's clastogenic effect can be postulated.

Preliminary analysis of the Seveso population data revealed a tendency toward an increase in the frequency of chromosomal aberrations in both acutely and chronically exposed persons. However, the differences (when compared with results from controls) were not statistically significant. The trend was only partially confirmed by the later analysis. As shown by the results of the variance analysis, there was a considerable subjective component in the scoring of the various types of aberrations; observer differences were often significant. However, the differences in the frequencies of chromosomal aberrations (both including and excluding gaps) between exposure categories did not become significant even after correction for observer variance.

This conclusion applies to results obtained both from 48- and 72-hour cultures. If the incidence of chromosomal aberrations in the exposed groups is significant after increasing the number of mitoses analyzed, the cytogenetic risk following the Seveso accident will not be higher for persons living in the contaminated area than for the ICMESA workers, who were not exposed to the toxic cloud. Czeizel and Kiraly (1976) conducted a similar study. In comparison to controls, there was a higher frequency of chromosomal aberrations in blood of workers employed at a herbicide-producing factory in Budapest, regardless of whether or not they had been directly involved in the chemical production.

Indication of the lack of an obvious effect of acute exposure to TCDD on the incidence of chromosome lesions in blood is provided by analyzing the overall frequencies of persons with aberrant cells in the various categories.

In conclusion, the combined cytogenetic findings in the three samples examined--hospital patients, interrupted pregnancies, and individuals from Seveso and ICMESA populations-- may justify continuing cytogenetic monitoring of selected samples of the Seveso population, even though they have not provided consistent evidence of chromosomal effects associated with TCDD exposure.

REFERENCES

Albertini, R. J., and R. De Mars. 1973. Detection and quantification of X-ray induced mutation in cultured diploid human cells. Mutat. Res. 18:199-224.

Bloom, A. D. 1972. Induced chromosomal aberrations in man. Pp. 99-112 in H. Harris and K. Hirschhorn, eds. Vol. 3, Advances in Human Genetics. Plenum Press, New York.

Czeizel, E., and J. Kiraly. 1976. Chromosome examinations in workers producing Klorinol and Buvinol. Pp. 239-256 in L. Bank, ed. The Development of a Pesticide as a Complex Scientific Task. Medicina, Budapest, Hungary.

Evans, H. J., and M. L. O'Riordan. 1975. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen test. Mutat. Res. 31:135-148.

Keck, M., and I. Emerit. 1979. The influence of culture medium composition on the incidence of chromosomal breakage. Hum. Genet. 50:277-283.

Schinzel, A., and W. Schmid. 1976. Lymphocyte chromosome studies in humans exposed to chemical mutagens. The validity of the method in 67 patients under cytostatic therapy. Mutat. Res. 40:139-166.

Schneider, E. L., E. J. Standbridge, C. J. Epstein, M. Golbus, G. Abbo, and G. Rogers. 1974. Mycoplasma contamination of cultured amniotic fluid cells: Potential hazard to prenatal chromosomal diagnosis. Science 184:477-480.

Simoni, G., L. Larizza, N. Sacchi, G. Della Valle, F. Dambrosio, and L. De Carli. 1979. Chromosome lesions in amniotic fluid cell cultures. Hum. Genet. 49:327-332.

Strauss, G. H., and R. J. Albertini. 1977. 6-Thioguanin resistant lymphocytes in human peripheral blood. Pp. 327-334 in D. Scott, B. A. Bridges, and F. H. Sobels, eds. Progress in Genetic Toxicology. Elsevier North-Holland Biomedical Press.

Tenchini, M. L., R. Giorgi, C. Crimaudo, G. Simoni, F. Nuzzo, and L. De Carli. 1979. Approaches to examination of genetic damage after a major hazard in chemical industry: Preliminary citogenetic findings on TCDD exposed subjects after Seveso accident. Pp. 301-317 in K. Berg, ed. Genetic Damage in Man Caused by Environmental Agents. Academic Press, New York.

Wasson, J. S., J. E. Huff, and N. Loprieno. 1978. A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutat. Res. 47 (2/4):141-160.

Zavola, C., P. Arroyo, R. Lisker, A. Carnevale, F. Salamanca, J. I. Navarrete, F. M. Jimenez, B. Blanco, V. Vasquez, J. Sanchez, and S. Canun. 1979. Variability between and within laboratories in the analysis of structural chromosomal abnormalities. Clin. Genet. 15:377-381.

DISCUSSION

DR. MILLER: Is there a plan to study sister-chromatid exchange (SCE) in the chronically exposed people?

DR. DE CARLI: Yes. We are studying SCE's in individuals from the three categories, including the ICMESA workers who showed exceedingly high frequencies of chromosomal aberrations in the first analysis. We selected some 30 subjects from the three categories and matched controls. We examined SCE's and unscheduled DNA synthesis, and again, chromosomal aberrations. The results are being analyzed now.

DR. DARDANONI: The Epidemiological Commission of Seveso concluded that the study of frequency in a population of mixed exposure indicators may underestimate the real presence of damage because (1) what might be considered a homogeneous group of 163 people may be a mixture with different exposure conditions; (2) high intralaboratory variation may obscure the existence of aberration; and (3) inter-group variation may exist because not everyone had the same exposure. Could the data be reexamined along two lines? One, to confirm in the same individuals the stability of the gaps; and two, to determine if the data can be petitioned according to exposure indicates?

DR. DE CARLI: Yes, but we also are monitoring with different parameters the individuals from the three categories that showed the highest values. We can, though, both reexamine the old samples and examine the selected sample in successive analyses.

DR. MOORE: In Zone A, 163 people were heavily exposed and some developed frank chloracne. Have they been separated in the study of chromosome gaps or other aberrations from those who still might be in an exposed area but failed to show chloracne or enlarged liver?

DR. DE CARLI: Yes, we selected a sample of subjects showing chloracne, but they were not from Zone A; they were from Zones B and R. The differences were not significant in this sample when compared to nonchloracne cases.

DR. MILLER: Can you predict the likelihood of chromosomal effects on the basis of the chemical structure or biologic tests that have been made previously with TCDD? Would you expect TCDD to damage chromosomes?

DR. DE CARLI: The available data from chromosome studies are inconsistent. Results differ, even within the same group of investigators. Negative results and occasionally positive results have been reported using bone marrow of rats continuously treated with TCDD.

DR. MALTONI: Do you explain the difference between laboratories as resulting from the different samples or from the criteria used to characterize the lesions?

DR. DE CARLI: The differences can be ascribed both to differences in criteria for classifying aberrations and to sampling effects.

DR. MALTONI: Did you have any type of collegial discussion to clear up the difference?

DR. DE CARLI: Yes, with the conclusion that probably there is a sampling fluctuation. There is no doubt, however, that one of the laboratories tends to give higher estimates than the others. But this effect is systematic, and we can correct for it.

Cytochrome P-450 Induction By 2,3,7,8-Tetrachlorodibenzo-p-dioxin, Polychlorinated Biphenyls, and Polybrominated Biphenyls

Robert Neal[1]

The major enzyme systems involved in metabolizing xenobiotic compounds are the monooxygenase systems containing cytochrome P-450. Recent studies have indicated the presence of numerous species of cytochrome P-450 in the organs of a single animal; for example, the liver of the Sprague-Dawley rat contains at least six. These various species of cytochrome P-450 are inducible, to varying degrees, by a large number of hydrophobic compounds, which can be divided into two classes. One class contains the compounds that induce various species of cytochrome P-450 in a manner similar to phenobarbital; the second contains the compounds that induce various species of cytochrome P-450 in a manner similar to 3-methylcholanthrene.

The chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is also a potent inducer of species of cytochrome P-450 that are similar to those induced by 3-methylcholanthrene.

Various polychlorinated biphenyl (PCB) and polybrominated biphenyl (PBB) isomers are metabolized by the cytochrome-P-450-containing monooxygenase systems. The biologic half-life of various isomers of PCB's and PBB's appears to be related to the ability of the cytochrome P-450 monooxygenase system to metabolize them. The results of various studies indicate that PCB and PBB isomers with two or more adjacent, unsubstituted carbons are readily metabolized by the cytochrome P-450 monooxygenase systems.

Recent work in various laboratories has indicated that TCDD is metabolized in the rodent to more polar metabolites as shown by radioactivity excreted in urine and bile of hamsters that were given [^{14}C] - or [^{3}H] - TCDD.

Xenobiotic metabolism is altered after exposure to environmental chemicals. Polycyclic aromatic hydrocarbons are particularly potent inducers of the enzymes involved in metabolizing xenobiotics, and cytochrome P-450 is the most important of these enzymes. Cytochrome P-450 is involved in the metabolism of numerous xenobiotic compounds and is particularly important in terms of biotransformation of potentially toxic compounds.

---

[1] Chemical Industry Institute of Toxicology, Research Triangle Park, N.C.

The term "cytochrome P-450" describes a component of the so-called drug-metabolizing enzyme system that is predominantly found in the liver; however, it is also present in other tissues in smaller amounts. It gets its name from the absorption maximum of the carbon monoxide complex with the reduced form of this heme enzyme. Thus, the carbon monoxide complex with the reduced form of this enzyme has a peak absorption at 450 nm.

The mechanism of the metabolism of substrates by the cytochrome-P-450-containing enzyme system is as follows: First, the substrate is bound to the enzyme. Next, two electrons, supplied by the reduced pyridine nucleotide NADPH, are transferred by way of a flavoprotein to cytochrome P-450. Molecular oxygen then binds to the heme iron of the reduced cytochrome P-450. In a final series of reactions, one of the atoms of the molecular oxygen is introduced into the substrate to produce an oxygenated substrate. The other oxygen atom receives the two electrons originally donated by NADPH and, in the presence of two protons, is converted to water. This enzyme system is variously called the drug-metabolizing enzyme system, the P-450 monooxygenase enzyme system, and the mixed-function oxidase enzyme system.

One of the interesting developments in recent years is the discussion of the heterogeneity of cytochrome P-450 in animal organisms. Initially, it was believed that cytochrome P-450 was a single enzyme. Then, emerging data began to suggest that there were probably two species of this enzyme. Studies had shown that the spectral properties of the enzyme induced by phenobarbital were different from those induced by 3-methylcholanthrene. The species induced by phenobarbital had a γ_{max} at 450 nm and was called cytochrome P-450. The species induced by 3-methylcholanthrene had a γ_{max} of its carbon monoxide complex with the reduced enzyme at 448 nm, and thus was called cytochrome P-448.

More recent data suggest that there are multiple cytochrome P-450 enzymes present in biologic tissues. As many as 8 to 12 different species of cytochrome P-450 can be identified in rat liver by using various inducers of the cytochrome P-450's such as phenobarbital or 3-methylcholanthrene. These various species of cytochrome P-450 differ both in molecular weight and spectral properties. The biologic reason for the existence of so many cytochrome P-450 enzymes is unclear. Many may be present because of evolutionary processes. For example, new forms may have developed with the capability of metabolizing the different nonpolar exogenous substrates to which our ancestors were exposed in the changing environment.

The various species of cytochrome P-450 do have somewhat different substrate specificity and therefore can metabolize some compounds more readily than others. However, in almost all cases there is a great deal of overlap in the ability of the various species of cytochrome P-450 to metabolize various nonpolar compounds.

Polychlorinated biphenyls (PCB's) and polybrominated biphenyls (PBB's) are potent inducers of the activity of the cytochrome P-450 monooxygenase systems. It recently became clear that if one exposes animals to Arochlor 1254 (which is in fact a mixture of compounds), the activity of a number of species of cytochrome P-450 is induced.

As noted previously, there are two types of inducers of cytochrome P-450: the phenobarbital-like inducers and compounds that induce species of P-450 with a spectral maximum of the reduced carbon monoxide complex of approximately 450 nm. The 3-methylcholanthrene-like compounds induce cytochrome P-450's with a reduced carbon monoxide spectral maximum that averages about 448 nm. Experiments with PCB's and PBB's have shown a mixed-type induction, that is, both types of cytochrome P-450 were induced.

Investigations of individual isomers of PCB's have provided an understanding of why PCB's and PBB's are mixed inducers. Upon examination of the inducing properties of 2,4,5,2',4',5'-hexachlorobiphenyl, one observes a species of cytochrome P-450 with a spectral maximum of 450 nm. However, with 3,4,5,3',4',5'-hexachlorobiphenyl, the spectral maximum averages approximately 448 nm. Thus, the mixed-type induction observed for commercial PCB's and PBB's undoubtedly results from the fact that the mixture of polychlorinated biphenyls contains different structural isomers of the polyhalogenated biphenyls that can induce different spectral types of P-450.

Dr. Joyce Goldstein, at the National Institute of Environmental Health Sciences (NIEHS), has identified various individual PCB isomers that induce cytochrome P-450 and cytochrome P-448 (Goldstein et al., 1977). In addition, she has shown that individual PCB isomers can be strong, moderate, weak, or inactive inducers. The isomers that are more highly chlorinated and that are chlorinated at positions ortho to the ring juncture are strong P-450 inducers. The more highly chlorinated these compounds are, the less readily they are metabolized and the more persistent they are in experimental animals. This is probably an important factor in their ability to be either strong or weak inducers of cytochrome P-450.

3,4,3',4'-Tetrachlorobiphenyl and 3,4,5,3',4',5'-hexachlorobiphenyl are among the few PCB isomers that induce P-450 enzymes with a reduced carbon monoxide $_{max}$ at 448 nm, rather than 450 nm. They are also the most toxic of the PBC isomers so far examined (McKinney *et al.*, 1976). These two PCB isomers can assume a planar structure since they are not chlorinated in the position ortho to the ring juncture. Isomers that are chlorinated ortho to the ring juncture cannot be planar because the rings have to rotate to accommodate the steric properties of the halogen atom. Both 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the corresponding tetrachlorodibenzofuran are planar molecules and strong P-448 inducers.

PCB's are metabolized by the cytochrome-P-450-containing enzyme systems. The PCB isomers that are the best substrates for such systems have two adjacent carbons that are not substituted with chlorine atoms. Fully chlorinated biphenyl, as well as those biphenyls containing 9, 8, and 7 chlorines and some compounds containing 6 chlorines, are poor substrates for the P-450-containing monooxygenases. TCDD does not have two adjacent unchlorinated carbons. Therefore, it is not a good substrate for the cytochrome P-450 enzyme systems. Until quite recently, it was believed that TCDD was not metabolized by mammalian enzymes. However, recent data indicate that TCDD is metabolized in certain rodent species (Olson *et al.*, 1980; Poiger *et al.*, 1979).

The single dose LD_{50} of TCDD is quite variable among species. For example, in the guinea pig the LD_{50} is approximately 1 μg/kg; in the rat, it ranges from 20 μg/kg to 40 μg/kg; in the chicken, from 25 μg/kg to 50 μg/kg; in the mouse, from approximately 100 μg/kg to 300 μg/kg; and in the monkey, it is approximately 70 μg/kg. The LD_{50} of TCDD in the Golden Syrian hamster has been found to be greater than 3,000 μg/kg when administered by intraperitoneal injection, and approximately 1,000 μg/kg when administered orally. Thus, of the species examined so far, the hamster is the most resistant to the toxicity of TCDD.

The half-life of excretion of TCDD has been examined in various rodent species. In the guinea pig and rat, the half-life is approximately 30 days. In the hamster, it is about 12 to 15 days. Thus, hamsters excrete TCDD much more rapidly than does the guinea pig or the rat.

Hamsters given 600 μg/kg [^{14}C] TCDD by intraperitoneal injection excrete approximately 30% to 35% of the radioactivity in urine. In contrast, the rat excretes only about 2% to 5% by this route. These and other data suggest that radioactivity in the urine of hamsters and, perhaps, rats is in the form of metabolites. TCDD metabolism has been examined in the hamster in greater detail. Examination for urinary metabolites using high-pressure liquid chromatography (HPLC) has revealed that all the radioactivity in urine elutes from the column as metabolites of TCDD. No TCDD itself could be detected in the urine.

The radioactivity excreted in bile following administration of [^{14}C] TCDD to hamsters was also examined. Again, none of the radioactivity excreted in hamster bile appeared as TCDD, but, in fact, occurred as a major metabolite and as a number of minor metabolites of TCDD.

The nature of the radioactivity remaining in liver following administration of [^{14}C] TCDD to hamsters was also examined. The radioactivity in liver was unchanged TCDD, suggesting that TCDD is very rapidly excreted after metabolism.

If the major peak of radioactivity excreted in bile is treated with β-glucuronidase and rechromatographed, all the radioactivity is eluted from the HPLC column at a later time, suggesting that the major product excreted in bile is a glucuronide derivative of TCDD. This implies that TCDD is being hydroxylated and a glucuronide formed before excretion in bile.

An examination of TCDD metabolism _in vitro_ (using hamster microsomes) shows evidence of covalent binding of radioactivity from [^{14}C] TCDD to macromolecules of the microsomes. Little or no binding is observed when TCDD is incubated with boiled microsomes or with microsomes in the absence of an NADPH-generating system. When isolated primary hepatocytes from hamsters are used, TCDD is metabolized to products that accumulate in the incubation medium. The major metabolite formed using this hepatocyte system appears to be the same glucuronide derivative detected in bile.

In summary, the P-450-containing enzyme systems are important in the metabolism of foreign compounds. They are strongly induced by chlorinated aromatic compounds. Particularly potent inducers of these enzymes are TCDD, the dibenzofurans, and 3,4,5,3',4',5'-hexachloro- or hexabromobiphenyl.

REFERENCES

Goldstein, J. A., P. Hickman, H. Bergman, J. D. McKinney, and M. P. Walker. 1977. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. Chem. Biol. Interact. 17:69-87.

McKinney, J. D., K. Chae, B. N. Gupta, J. A. Moore, and J. A. Goldstein. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,78-tetrachlorodibenzofuran in chicks. I. Relationship of chemical parameters. Toxicol. Appl. Pharmacol. 36:65-80.

Olson, J. R., T. A. Gasiewicz, and R. A. Neal. 1980. Tissue distribution, excretion, and metabolism of 2,3,7-tetrachlorodibenzo-p-dioxin (TCDD) in the golden Syrian hamster. Toxicol. Appl. Pharmacol. 56:78-85.

Poiger, H., and Ch. Schlatter. 1979. Biological degradation of TCDD in rats. Nature 281:706-707.

DISCUSSION

DR. MILLER: You showed the hamster to be relatively resistant to TCDD. Does this have foreseeable implications for the treatment of people--to make them react more like hamsters?

DR. NEAL: The interesting thing to determine is whether humans are more like hamsters or more like guinea pigs. We could, for example, use an *in vitro* metabolizing system to compare the abilities of rat, hamster, and human tissue to metabolize TCDD. But the key question is whether the hamster is more resistant to TCDD because it metabolizes it more rapidly or because of other factors. The most popular theory concerning TCDD's acute toxicity is that TCDD binds to a receptor in cytosol. This receptor-TCDD complex is then translocated into the nucleus, followed by the coordinate derepression of the synthesis of a number of enzymes. The increase in enzyme synthesis caused by this derepression is postulated to cause TCDD's acute toxicity.

An examination of hamster liver reveals the presence of about the same level of receptor as seen in rat; however, as noted previously, the hamster is orders of magnitude less susceptible to TCDD toxicity than is the rat. Thus, toxicity does not appear to be related to the level of receptor in livers of the two species. I don't know whether the increased rate of metabolism or the increased rate of TCDD excretion explains why the hamster is so resistant to TCDD's acute toxicity. But it is clearly an interesting avenue to pursue.

DR. MURPHY: Is there any relationship between Goldstein's observation of apparent correspondence between inducibility of cytochrome P-450 monooxygenase activity and toxicity of these compounds to various species, especially the hamster?

DR. NEAL: TCDD does induce enzymes in the hamster. I don't know that we can say "easily," because we haven't compared the rat and hamster at the same dosage level to see if we get the same degree of induction of cytochrome P-450 or other enzymes in both species. However, 3-methylcholanthrene can induce the same enzymes as TCDD, but the toxicity seen with TCDD is not evident in the case of 3-methylcholanthrene. Therefore, there is no reason to believe that the induction of the enzymes by TCDD is responsible for the acute toxicity. There may be an induction of the activity of some unknown enzymes or an increase in synthesis of some unknown proteins, perhaps a membrane protein, which could lead to toxicity.

DR. GARATTINI: If you give the same dose, for example, a relatively low dose of 10 μg/kg to rats and hamsters, do you get the same kind of induction for P-448?

DR. NEAL: We need to look at that--that is, we need to compare the ability of TCDD to induce P-448 in the hamster and other species.

DR. GARATTINI: That would be extremely important in determining whether the sensitivity of the system or the metabolism is involved. Do you have any suggestion about the preferential site for metabolism of TCDD? Which chlorine is going out?

DR. NEAL: We don't know. We now have about 3 g of the major TCDD metabolite isolated in a reasonably pure form, and we are going to perform mass spectral analysis on it. It should be easy to tell whether we have lost a chlorine.

DR. GARATTINI: It would be difficult to find the position.

DR. NEAL: Yes. However, if all four chlorines remain in the metabolite, then the four unsubstituted positions are equivalent. In other words, if all four chlorines are still there, then all four unsubstituted positions would be equivalent relative to hydroxylation. Thus, the key questions are whether the tricyclic structure is still intact and whether all four chlorines remain in the metabolite. If that is the case, then the identification of the metabolite should be fairly straightforward.

DR. GARATTINI: We tried to determine if there was covalent binding to proteins with liver microsomes from the rat and mouse. We could find no evidence that agrees with what you found.

As to the half-life of TCDD in hamsters, you tried to explain the reduced toxicity by the relatively shorter half-life; however, in mice, the half-life is still shorter. Yet, the toxicity is much higher, on the order of 50 μg/kg.

DR. NEAL: I wasn't aware that the half-life studies in the mouse had been done. You carried out these studies?

DR. GARATTINI: Yes. Apparently TCDD is excreted, but we couldn't find any metabolite excreted as such.

DR. NEAL: About covalent binding, we do see it with the hamster microsome system, but not with the rat.

DR. MILLER: In Seveso, where there was exposure to TCDD, which induces P-450, would you expect newborn infants to have a shorter duration of neonatal jaundice because of the induction of the enzyme?

DR. NEAL: It would probably depend on whether the species of P-450 induced will in fact work on bilirubin. Different species of P-450 display different substrate specificity. The species induced by TCDD may not, in fact, work on bilirubin. I don't know the answer to your question.

DR. MILLER: If the people still had TCDD in their bodies, is there a drug test to measure the influence of the TCDD on the metabolism of the drug?

DR. NEAL: No. There are so many compounds--literally hundreds of them--known to induce cytochrome P-450 or P-448 or this whole spectrum of P-450 enzymes; a number of these compounds are drugs. We are exposed to many inducers on a daily basis because they are present in our environment, including our food. Therefore, I think a drug metabolism test would be useless in trying to assess a body burden of TCDD. The only way to identify TCDD in tissue is to detect it analytically.

DR. GARATTINI: A metabolism test in children might not definitively measure aryl hydrocarbon hydroxylase (AHH), but it certainly could indicate a strong increase.

DR. NEAL: Except that TCDD is not a particularly good inducer of P-450 in a quantitative sense. The activity of P-450-like reactions is actually lower in TCDD-treated animals than in untreated ones. Thus, in TCDD-treated rats, benzamphetamine-demethylase activity is actually decreased whereas benzo(a)pyrene activity is increased.

If people with TCDD ate cabbage or brussels sprouts the day before the enzyme activity was measured, you would get a false result. Certain foods contain some very potent inducers of those AHH enzymes.

DR. GARATTINI: In given control conditions, perhaps in children, because they are not smokers, AHH might serve as an indicator if you find a substantial increase.

DR. NEAL: You would probably have to have a great amount of data on the level in normal individuals before you could attribute an increase in AHH to the presence of TCDD.

SPEAKER (UNIDENTIFIED): What fraction of the material that was radiolabeled was excreted as glucuronide versus the fraction bound covalently to cellular macromolecules?

DR. NEAL: If we do the experiments with either [^{14}C]- or [^{3}H]-labeled TCDD (1,6-[^{3}H] TCDD), we get covalent binding of radioactivity. I have shown data using [^{14}C]-labeled TCDD. If we use 1,6-[^{3}H]-TCDD, we obtain an extra peak off the HPLC columns. This peak elutes very early and appears to be tritiated water. These data also suggest that P-450 is hydroxylating TCDD in the 1, 4, 6, or 9 position and, as a result, tritium is lost as $^{3}H_2O$.

Thus, we see the binding both of [^{14}C]- and tritium-labeled TCDD. Both ^{14}C and tritium are observed in the metabolites isolated from urine and bile, although an extra peak is observed in the HPLC columns from urine and bile of animals administered [^{3}H]-TCDD. The compound appears to be tritiated water. You can evaporate off the radioactivity in that HPLC peak.

DR. MURPHY: Couldn't you use a microsomal enzyme assay as suggestive evidence of exposure to TCDD or related compounds?

DR. NEAL: No, because so many compounds induce P-450. Also, I don't think you can use an *in vivo* test of drug metabolism. In some methods, you give a drug you know is metabolized by an enzyme system and look at excretion products in urine. In this case, the test would be hard to control. It would be difficult to determine a normal level in a human population. Fred Guengerich of our center has been studying human cytochrome P-450's, isolating and purifying them from liver. He has looked at about 20 human liver samples and purified enzymes from some of them. The amounts of enzyme he sees are quite variable. The P-450 monooxygenase activities appear to be quite variable, as is the substrate specificity. Therefore, I don't believe you can use an enzymatic assay to analyze body burden of TCDD.

Hepatic Toxicity of TCDD

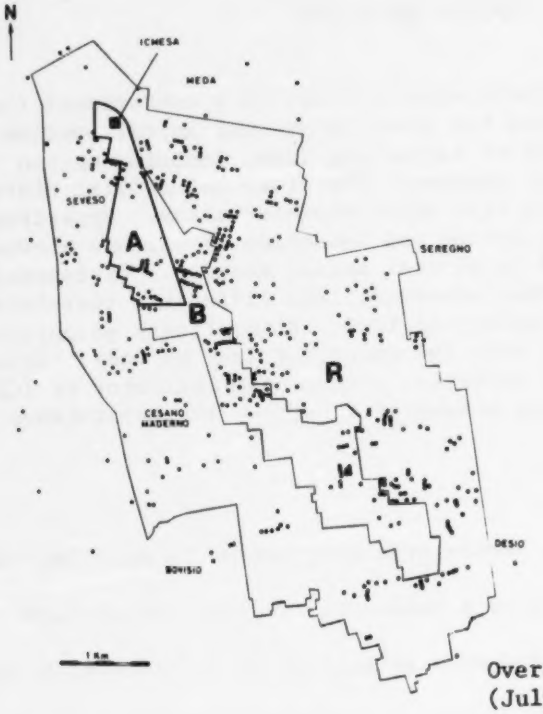
Silvio Garattini[1]

2,3,7,8-Tetrachlorodibenzodioxin (TCDD) is a contaminant that shows an exceptional tropism for liver in several animal species. The chemical persists in liver for a long time, inducing marked morphologic and biochemical changes. The liver subcellular distribution of TCDD changes with time after administration. Hypertrophy of the reticuloendothelial system and induction of various microsomal enzymes have been reported in several animal species. Differences among mouse strains have been observed; this difference correlates with the immunosuppresive effect of TCDD. TCDD affects porphyrin metabolism in rats treated with low doses for long periods. Changes in porphyrin excretion may represent a sensitive indicator of TCDD exposure. Studies have also attempted to affect the persistence of TCDD in liver.

Animals living in the Seveso area were useful in defining the contamination and for starting a number of studies. At the time of the accident, some 81,000 domestic animals lived in Zones A, B, and R. Mortality, mostly among rabbits and poultry, started some days after the accident, rising markedly within the first 2 weeks. By the end of August 1976, 3,281 dead animals had been recorded. Figure 1 shows overall animal mortality figures and the location of farms where deaths occurred. Rabbit mortality is given in Table 1. Deaths occurred on 75% of the farms in Zone A, on 22% of the farms in Zone B, and on 14% of those in Zone R. In Zone A, 31.9% of the rabbits died, 8.8% in Zone B, and 6.8% in Zone R. On farms where rabbits died, the percentage of mortality was 42% in Zone A, 23% in Zone B, and 16%

---

[1] Istituto di Ricerche Farmacologiche "Mario Negri," 62, Via Eritrea, 20157 Milan, Italy.


N
ICMESA
MEDA
SEVESO
SEREGNO
A
B
R
CESANO MADERNO
DESIO
BOVISIO
1 km

Overall animal mortality
(July 10 - August 31, 1976

Animal	No. of dead animals over total
Rabbits	2,062/24,885
Other small farmyard animals	1,219/55,545
Cattle	0/349
Horses	0/ 49
Pigs	0/233
Sheep	0/ 21
Goats	0/ 49

FIGURE 1. Distribution of animal mortality in the Seveso area; ● = Farms with mortality. On the basis of soil analysis for TCDD three zones were designated:
A = TCDD levels in soil from <0.75 to 5,477 $\mu g/m^2$
B = TCDD levels in soil from <0.75 to 43.8 $\mu g/m^2$
R = TCDD levels in soil from <0.74 to 5 $\mu g/m^2$

Data from Servizio Veterinazio della Lombardia

TABLE 1

Rabbit Mortality on Farms in the Contaminated Zones, July 10 - August 31, 1976[a]

	No. of Rabbits			No. of Farms in Area			No. of Rabbits on Farms Where Deaths Occurred		
Zone[b]	Total	Dead	%	Total	Farms with deaths	%	Total	Dead	%
A	1,089	348	31.9	45	34	75.5	825	348	42
B	4,814	426	8.8	303	67	22.1	1,801	426	23
R	18,982	1,288	6.8	1,398	208	14.9	7,783	1,288	16
Total	24,885	2,062	8.3	1,746	309	17.7	10,409	2,062	19.8

---

[a] Data from Servizio Veterinario della Lombardia.

[b] See Figure 1 for soil contamination levels in Zones A, B, and R.

ICMESA (Industrie Chimiche Meda Societa Azionaria) factory, and there is strong evidence that mortality was directly related to how close to ICMESA fresh fodder was gathered. Farms where rabbits were fed commercial feed and/or fodder collected before the accident or far from ICMESA had lower mortality rates. TCDD was analyzed in samples from domestic and wild animals that had died or been slaughtered for safety reasons to monitor contamination and to measure the accumulation of TCDD in living organisms (Table 2). Milk collected 2 weeks after the accident (from cows living close to the chemical plant and fed contaminated fodder) contained up to 8 g of TCDD per liter. Rabbits that died (or that were slaughtered within 1 month of the accident) had levels of TCDD in liver ranging from 0.25 ng/g to 633 ng/g. Twenty percent of 900 autopsied rabbits had a syndrome characterized by substernal and retrosternal edema, hemorrhagic tracheitis, pleural serous hemorrhage, and dystrophic lesions of hepatic tissue. TCDD played an important part in this toxic syndrome, as indicated by the presence of large amounts of the chemical in the liver of many of the animals. Similar liver alterations and TCDD concentrations (100 ng/g) were found in rabbits that died or were killed within 1 month of receiving a single oral dose of 60-120 μg/kg of TCDD.

Rabbits were believed to be a reliable model of the risk of TCDD exposure because the chemical accumulates in their tissues and becomes detectable in their liver after ingestion of the compound or the environmental exposure. At the end of 1976, all rabbits in zones A, B,

TABLE 2

Overall TCDD Analyses of Animals from Contaminated Zones and Surrounding Areas[a]

Animals	No. of Samples	% TCDD Positive	Maximum Detected Level (ppb)
Farm animals			
Rabbit[b]	698 (all	62	633
Poultry	83 liver)	42	24
Cattle	43	5	10
Horses	12	17	88
Pigs	13	0	-
Goats	25	68	1,253
Guinea pigs	4	0	-
Cats	1	0	-
Wildlife			
Hares	6 (liver)	67	13
Field mice	14 (whole body)	100	49
Rats	1 (pool of four livers)		28
Earthworms	2 (pool)		12
Frogs	1 (liver)		0.2
Snakes	1 (liver)		3
Cow's milk	103	26	8
Total	1,007		

---

[a] Gas chromatography-mass fragmentography was used for detection, identification, and measurement of TCDD in tissues (limit of detection 0.25 ng/g). A detailed description of the method is reported in Fanelli *et al.*, 1980.

[b] Figures include rabbits kept in the special test plots of contaminated ground for experimental purposes.

and R were killed, and pilot breedings were established in Zone R in fields with known soil contamination (approximately 1.5 $\mu g/m^2$). TCDD was analyzed in liver from these rabbits after periods ranging from 60 to 300 days. Samples of rabbits from farms outside Zone R were also analyzed. Table 3 shows the percentages of positive samples from 1976 to 1979. In pilot breedings and surrounding areas, during the years 1978 and 1979, only few animals of those examined were found contaminated and the concentration of TCDD in their liver never exceded 750 pg/g.

The study was helpful for several reasons: The animal data confirmed the division into three geographic areas and uncovered some contamination outside Zone R. Pilot breedings showed that traces of TCDD could still be found in liver of rabbits kept in areas with a soil contamination of the order of 1.5 $\mu g/m^2$.

Studies are still in progress on the relationship between liver pathology and liver concentrations of TCDD. In the rabbit, as in the rat and mouse, the liver is not only the site of accumulation of TCDD, but also is more severely altered than other organs (Gupta et al., 1973; McConnell et al., 1977; Rose et al., 1976). When rabbits were treated orally with [^{3}H]-TCDD (2.5, 25, and 500 ng/kg every other day) for 30 days, the levels of [^{3}H]-TCDD almost reached a steady state (Table 4) as predicted by the rate of elimination of TCDD from liver (half-life about 7 days). Microscopic

TABLE 3

Presence of TCDD as Percentage of Positive Samples in Liver of Rabbits from Contaminated Zones, 1976-1979[a]

Time of Sampling	Zone A	Zone B	Zone R	Surrounding Areas
July-August 1976	97 (29)	92 (37)	75 (122)	27 (22)
September-December 1976	98 (42)	91 (11)	77 (127)	43 (7)
1977	all animals killed		93[b] (31)[b]	12 (41)
1978	-	-	5[b] (59)[b]	15.9 (44)
1979	-	-	2.4[b] (42)[b]	2.8 (70)

---

[a] Figures in parentheses are numbers of animals analyzed for TCDD. For the determination of TCDD in biologic samples, see Fanelli et al., 1980. The sensitivity of the method was 0.25 ng of TCDD per gram.

[b] In 1977, except for a small number of pilot breedings set up in Zone R, there were no more animals in Zones A, B, or R. These figures refer to the pilot breedings. Samples were also taken from farms located outside Zone R (surrounding areas).

TABLE 4

Concentration of [^{3}H]-TCDD in Rabbit Liver[a]

Oral Dose Every Other Day, ng/kg	^{3}H-TCDD in the Liver, ng/g			Theoretical Steady State
	8 Days	22 Days	30 Days	
2.5	– [b]	0.15 ± 0.03	0.12 ± .008	0.16
25	0.8 ± 0.09	0.59 ± 0.14	0.92 ± 0.12	1.4
500	7.9 ± 1.7	27.7 ± 1.7	25.3 ± 2	23.4

---

[a] Rabbits were given [^{3}H]-TCDD orally at a dosage of 2.5 ng (0.4 Ci), 25 ng (4 Ci) and 500 ng (5.9 Ci) per kilogram every other day for 30 days. Three or four rabbits were killed after 8, 22, and 30 days. A portion of the livers was homogenized and its radioactivity extracted, identified by thin-layer chromatography, and determined by liquid scintillation counting.

[b] Not measured.

examination of the liver showed alterations related to the liver concentrations of [^{3}H]-TCDD (swelling of cells, fatty degenerations, cell necrosis), and also showed that the time of exposure to TCDD was very important. For example, liver with 7.9 ng of TCDD per gram, dissected from animals exposed for 8 days to 500 ng/kg every other day, had alterations similar to those of liver with 0.6 ng of TCDD per gram, dissected from animals exposed for 30 days to 25 ng/kg every other day.

Over time, TCDD can change location within the cell as seen by administering TCDD to mice and then looking at different subcellular fractions of the liver (nuclear, mitochondrial, microsomal, soluble). Figure 2 shows that the distribution profile of TCDD in subcellular fractions 7 days after receipt of a single dose of TCDD is very similar to that observed when TCDD is added *in vitro*. The largest percentage is in the nuclear fraction, and smaller amounts are in the mitochondrial, microsomal, and soluble fractions. However, 14 days after TCDD injection, the subcellular distribution profile is completely different. There is markedly less TCDD in the nuclear fraction, but more in the mitochondrial and microsomal fractions. This shift of TCDD in the cells occurs without substantial changes in total levels of TCDD in the liver. Thus, even when the total TCDD level is the same, its subcellular distribution can differ.

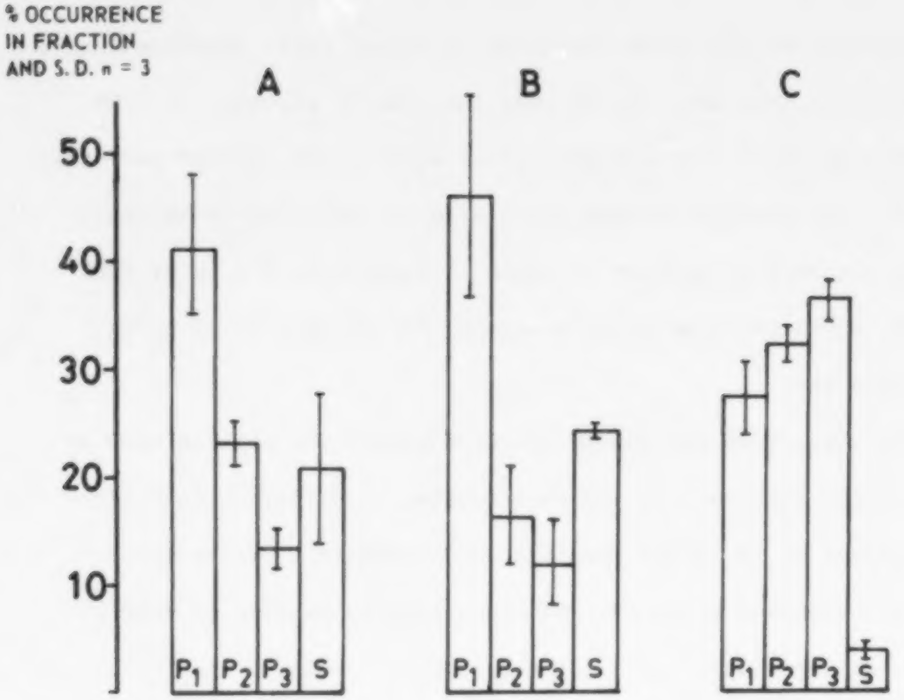


FIGURE 2. TCDD distribution in mouse liver subcellular fractions. Male C57BL/6J mice were injected intraperitoneally with 4 mCI, 25 μg/kg[^{3}H]-TCDD and were killed either 7 (b) or 14 (c) days later. [^{3}H]-TCDD (88 ng/g) was added to livers from nonintoxicated mice (A). Tissues were homogenized in five volumes of potassium chloride, 1.15% buffer plus 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 20 mM, pH 7.5, and subcellular fractions were prepared according to Lucier et al. (1973). P_1 (nuclear), P_2 (mitochondrial), and P_3 (microsomal) fractions were the sediments of centrifugations 670, 10,000, and 105,000 xg, respectively, and S (soluble fraction) was the high speed supernatant. Radioactivity in fractions was assayed (see Table 9). Occasional chromatography did not reveal evidence of biotransformation of TCDD in any fraction. Total liver TCDD concentrations (ng/g ± S.D.) were 103.9 ± 14.0 and 95.0 ± 25.4 for B and C, respectively. Three livers were processed individually for each group. From unpublished data of P. Coccia, T. Croci, and L. Manara, 1980.

Continuous treatment with TCDD causes porphyria in both laboratory animals and humans (Goldstein et al., 1973; Poland et al., 1971). In mice, porphyria is inherited, as is the induction of aryl hydrocarbon hydroxylase (AHH) (Jones and Sweeney, 1977, 1980). However, it was not known what dose of TCDD, given to animals continuously, had no porphyrogenic effect and caused no microsomal enzyme induction. Nor was it clear whether evaluation of the pattern of excretion of porphyrins throughout TCDD administration was a sensitive parameter for monitoring TCDD exposure. TCDD was given to female rats at three dose levels--10, 100, and 1,000 ng/kg, once a week for 45 weeks. At the end of treatment, body weights were unchanged in animals receiving the two lower doses, but an 8% loss was observed in animals receiving the highest dose. Liver weight increased proportionally to the dose administered (19% increase with the lowest dose, corresponding to 1.4 ng of TCDD per kilogram per day).

The P-450 and mixed-function oxidase (MFO) activities are shown in Table 5. P-450 was raised only in animals receiving 1,000 ng/kg/wk. AHH was markedly increased at all doses. There was also a marked dose-dependent increase in the levels of 7-ethoxy-coumarin-O-deethylase (7-EC), which is known to be induced by pretreatment with both phenobarbital and 3-methylcholanthrene. Increases were noted at the lowest (4-fold) and highest (30-fold) doses. The same trend of induction was observed when enzymatic activities (per nanomole of cytochrome P-450) were calculated. The porphyrogenic effect of TCDD was marked in animals

TABLE 5

Liver Microsomal Cytochrome and Mixed-Function Oxidase Activity After Chronic TCDD Treatment[a]

TCDD Dose (ng/g/week)	P-450 (nmol/mg protein	AHH (pmol/min/mg)	7-EC (pmol/min/mg protein)
None	0.56 ± 0.05	19.54 ± 8.80	131.27 ± 31.68
10	0.60 ± 0.06	143.25 ± 17.51[b]	583.43 ± 103.40[c]
100	0.68 ± 0.03	341.67 ± 12.4[b]	1,565.33 ± 25.14[b]
1,000	0.91 ± 0.13[c]	283.17 ± 50.25[c]	4,513.31 ± 1,105.99[b]

---

[a] Data from Cantoni *et al.*, 1981a. CD-COBS rats were killed after 45 weeks of treatment. Values represent mean ± S.E. of three or four animals. Cytochrome P-450, AHH, and 7-EC were determined as described by Omura and Sata (1964), Nebert and Gelboin (1968), Greenlee and Poland (1978), respectively.

[b] $p < 0.01$, student's t-test.

[c] $p < 0.05$, student's t-test.

treated with the highest concentration (1,000 ng/kg/wk) for 10 months. The onset and development of porphyria was monitored by measuring the levels of the various porphyrins in urine throughout the experiment. In all three treated groups, there was an increase in the absolute amount of coproporphyrin excreted during the 24-hour period. After 10 months, this increase was significant, even in animals receiving the lowest dose of TCDD (Figure 3).

The levels of other toxicologically important porphyrins excreted in urine were also measured. The amount of uroporphyrin was increased in animals receiving 100 and 1,000 ng of TCDD per kilogram per week. The heptacarboxylic porphyrin level was elevated only in animals receiving the highest dosage. Hexacarboxylic porphyrin was never detected in control animals, but was measurable in urine of animals receiving 1,000 ng/kg/wk.

In animals given the highest doses of TCDD, increases in the excretion of porphyrins with a high number of carboxyl groups were accompanied by changes in the relative percentage distribution of each porphyrin. The principal change is the inversion of the copro:uro ratio (from 6 to 8 months after treatment), as compared to that in control animals. Total urinary prophyrin levels were also elevated 10 months after treatment. The difference at the lowest dose of TCDD was significant (Table 6). This increase was not accompanied by any change in porphyrins in liver. The marked rise

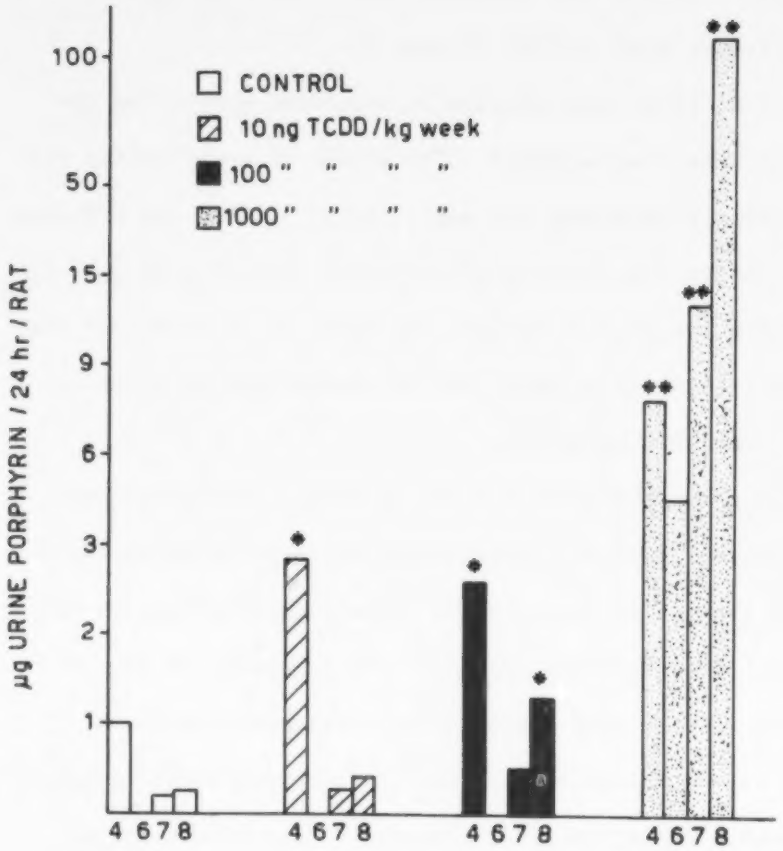


FIGURE 3. Urinary porphyrin excretion after TCDD treatment for 45 weeks. Values represent the mean of four animals. 4, coproporphyrin; 6, hexacarboxylic porphyrin; 7, heptacarboxylic porphyrin; 8, uroporphyrin. * = $p < 0.05$, student's t-test; ** = $p < 0.01$, student's t-test. Redrawn from Cantoni et al., 1981b.

TABLE 6

Hepatic Accumulation of TCDD and Total Porphyrin Content of Liver and Urine After TCDD Treatment for 45 Weeks[a]

TCDD Dose (ng/kg/week)	Liver TCDD (ng/g wet tissue)	Total Liver Porphyrins (ng/g wet tissue)	Total Urinary Porphyrins (nmol/24 hr)
None	0.05	1.59 ± 0.25	2.27 ± 0.49
10	1.55 ± 0.02	0.92 ± 0.20	5.55 ± 0.85[b]
100	4.74 ± 0.54	4.11 ± 2.96	7.62 ± 1.79[b]
1,000	30.70 ± 3.34	724.67 ± 2.67[c]	196.89 ± 63.14[c]

---

[a] Data from Cantoni *et al.*, 1981b. CD-COBS rats were killed after 45 weeks of treatment; values represent mean ± S.E. of three or four animals. TCDD content of liver and total liver porphyrins were determined as described by Fanelli *et al.* (1980) and Abbritti and De Matteis (1971, 1972). Total urinary porphyrins were measured from the sum of each individual porphyrin, as described by Doss (1970).

[b] $p < 0.05$, student's t-test.

[c] $p < 0.01$, student's t-test.

in porphyrins in the liver and urine of animals receiving the largest dose of TCDD reflects established prophyria.

In conclusion, a clear porphyrogenic effect was present only when the animals were treated with 1,000 ng of TCDD per kilogram weekly for 45 weeks. At the 10 ng dose, coproporphyrin excretion was altered, possibly an early sign of intoxication. When porphyria is established, the observed pattern of porphyrin excretion supports the hypothesis that activity of the liver enzyme uroporphyrinogen decarboxylase diminishes, as has been shown for hexachlorobenzene (Elder, 1978).

It is well known that TCDD causes atrophy of the thymus and of the thymus-dependent areas of lymphoid organs in the mouse, rat, guinea pig, and monkey (McConnell et al., 1978; Vos et al., 1973). When TCDD is administered during the perinatal period through maternal treatment (before or after birth), severe consequences are observed in the immune system and cell-mediated responses are inhibited (Vos and Moore, 1974). More recent studies (Mantovani et al., 1980; McConnell et al., 1978; Vecchi et al., 1980 indicate that a single dose of TCDD (1-30 $\mu g/kg$) administered orally or intraperitoneally to 6- to 8-week-old C57BL/6 mice does not reduce cell-mediated activities (splenocyte blastogenic response to mitogens, graft versus host reaction, macrophage, and natural killer-cell cytotoxic activities) on a per cell basis, but does reduce the number of splenocytes and macrophages, indicating general impairment of the immune response (Mantovani et al., 1980).

In contrast, the same dose levels produce a functional, dose-dependent inhibition of primary and secondary antibody production to T-dependent and T-independent antigens (sheep erythrocytes and pneumococcal polysaccharide-type III), as indicated by the smaller number of antibody-producing cells per 10^6 splenocytes (Vecchi et al., 1980). Immunosuppression is marked and long-lasting. Thirty micrograms per kilogram inhibits humoral responses by 80% up to 6 weeks after a single dose.

TCDD is also a potent inducer of AHH in several animal species (Kouri et al., 1974; Poland et al., 1974). It induces hepatic AHH in mouse strains such as DBA/2, AKR, and SJL/J, which are not susceptible to 3-methylcholanthrene and have been described as nonresponsive strains (Poland et al., 1974). Nonresponsive strains require dose levels of TCDD approximately 10 times higher than those needed by responsive strains (e.g., C57BL/6, C3H/He, BALB/c, and A/J) to induce the AHH system (Robinson et al., 1974). This enzyme complex is believed to play an important role in the biotransformation of some aromatic compounds, producing and/or destroying toxic metabolites. Thus, the immunosuppressive activity of TCDD was investigated in both responsive and nonresponsive mice. In addition to the humoral antibody production, thymus weight was used as parameter of TCDD exposure.

As shown in Table 7, 6 μg of TCDD per kilogram significantly reduced thymus weight in responsive mice (C57BL/6 and C3H/He) only, not in the nonresponsive strains DBA/2 and AKR. Moreover, the same dose level caused a marked inhibition of antibody humoral response (80-90%)

TABLE 7

Effect of TCDD on Primary Humoral Response in Different Mouse Strains[a]

Mouse Strain	TCDD (μg/kg)	PFC/Spleen $\bar{X} \pm 1$ S.E.	% Control
C57BL/6	0	27334 (24106 - 30993)	100
	1.2	10514 (8698 - 12708)[b]	38
	6	3723 (2666 - 5199)[c]	14
	30	915 (765 - 1095)[c]	3
C3H/He	0	108239 (105177 - 111390)	100
	1.2	37378 (30028 - 46402)[c]	35
	6	5383 (3562 - 8137)[c]	5
DBA/2	0	16413 (13973 - 19279)	100
	1.2	13583 (11522 - 16014)	83
	6	8362 (7630 - 9160)	50
	30	5852 (4168 - 8215)[b]	36
AKR	0	51185 (50176 - 52213)	100
	1.2	-	
	6	31682 (26040 - 38546)	61.9

---

[a] Unpublished data by A. Vecchi, A. Mantovani, M. Sironi, W. Luini, 1980. TCDD was injected intraperitoneally in acetone:oil solution (1:6), 7 days before antigen challenge (4.10^8 SRBC); the test was performed 5 days later.

[b] $p < 0.05$, student's t-test.

[c] $p < 0.01$, student's t-test.

in C57BL/6 and C3H/He mice. In DBA/2 and AKR mice, the response was reduced by only 40-50% (Table 8). These data support the suggestion that the different sensitivities of various mouse strains to AHH induction reflect a more general difference in susceptibility to TCDD toxicity (Poland and Glover, 1980).

In laboratories at the Istituto Ricerche Farmacologiche "Mario Negri", investigators are searching for a way to shorten the persistence of TCDD in the body and to reduce its toxic action (Coccia *et al.*, 1981). In these studies a single dose of [3H]-TCDD[1] was administered orally by stomach intubation or intraperitoneally to male C57BL/6J mice, weighing 18 to 21 g, which were maintained in controlled environmental conditions. The animals were fed diets containing different additives or standard powdered chow.[2] Controls received standard chow throughout the experiment. The amount of TCDD in the livers of the treated mice was measured days later by radioassay and liquid scintillation. Thin-layer chromatography (*n*-hexane:ethyl ether, 16:1; R = 0.80) of selected samples gave no evidence of TCDD biotransformation in any group of animals. Representative results are summarized in Table 9. After 14 days approximately 10% to 17% of the administered dose is recovered in the livers of animals fed the standard diet. The addition of 4% cholestyramine does not change the results. However, when 5% charcoal is added to the diet, the amount of TCDD present in the liver is substantially reduced. The concentrations of TCDD in the livers of animals receiving charcoal were as much as 60% lower than those in controls receiving only TCDD. The addition of cholic acid, which stimulates bile flow, also lowered the amount of TCDD in the liver.

---

[1] Kor Isotopics, USA, S.A. 51C:/mM, 90% radiochemically pure dissolved in acetone: corn oil, 1:6, 5 ml/kg.

[2] "Altromin R," A. Rieper, Italy.

In another experiment, mice were given charcoal 3 days after they received TCDD; thus, the mice absorbed most of the TCDD through the intestinal tract before the charcoal could act (Unpublished data by P. Coccia, T. Croci, and L. Manara, 1981.) Even so, there was a much lower percentage of TCDD in their liver than in a control group. Finally, two different preparations of charcoal reduced the amount of TCDD in liver after intraperitoneal administration of TCDD. Interestingly enough, the effect of these two preparations was further increased by the addition of 1% cholic acid to the diet. Additional experiments indicated that charcoal and cholic acid are effective in reducing the mortality of mice given lethal doses of TCDD.

TABLE 8

Effect of TCDD on Thymus Weight in Different Mouse Strains[a]

Mouse Strain	TCDD (μg/kg)	Thymus Weight (mg)	% of Control
C57BL/6	0	64.3 ± 2.9	100
	1.2	57.0 ± 4.0	89
	6	45.0 ± 4.3[b]	70
	30	24.8 ± 3.0[b]	38
C3H/He	0	36.8 ± 2.5	100
	1.2	29.3 ± 2.9	80
	6	22.3 ± 2.1[b]	60
	30	14.0 ± 2.2[b]	38
DBA/2	0	27.7 ± 2.2	100
	1.2	22.4 ± 2.9	81
	6	23.4 ± 3.5	84
	30	20.3 ± 4.5	73
AKR	0	54.7 ± 3.5	100
	6	40.0 ± 9.3	73

---

[a] Unpublished data by A. Vecchi, A. Mantovani, M. Sironi, and W. Luini, 1980. TCDD was injected intraperitoneally in acetone:oil solution (1:6) 12 days before killing.

[b] $p < 0.05$, student's t-test.

TABLE 9

Radioactivity in Liver of Mice Fed Chow with Different Additives Measured 14 Days After a Single Dose of $[^3H]$-TCDD was Administered[a]

	Dose Recovered in Liver, mean percentage ± S.D. (Number of animals)		
Diet	7.6 μg/kg Oral Dose[b]	11.0 μg/kg Intraperitoneal Dose[c]	12.5 μg/kg Oral Dose[c]
Standard chow	17.3 ± 3.2 (6)	19.2 ± 1.6 (6)	10.3 ± 0.8 (5)
5% Vegetable charcoal	6.3 ± 1.1[d] (6)	15.3 ± 2.6[e] (7)	5.6 ± 0.8[e] (5)
0.5% Cholic acid	13.1 ± 2.8[d] (6)	f	f
5% Animal charcoal	f	13.8 ± 1.3[d]	f
5% Vegetable charcoal plus 1% Cholic acid	f	10.9 ± 2.4[d]	f
5% Animal charcoal plus 1% Cholic acid	f	9.1 ± 1.1[d]	f
4% Cholestyramine	14.5 ± 1.7 (6)	f	f

---

[a] DATA from Coccia *et al.*, 1981.

[b] Diets with added test substances were given to mice immediately after TCDD dose.

[c] Diets with added test substances were given to mice 3 days after TCDD dose.

[d] $p < 0.05$, Duncan's new multiple test.

[e] $p < 0.01$, Duncan's new multiple test.

[f] Not investigated.

REFERENCES

Abbritti, G., and F. De Matteis. 1971-1972. Decreased levels of cytochrome P-450 and catalase in hepatic porphyria caused by substituted acetamides and barbiturates. Importance of the allyl group in the molecule of the active drugs. Chem. Biol. Interact. 4:281-286.

Cantoni, L., M. Salmona and M. Rizzardini. 1981b. Porphyrogenic effect of chronic treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin in female rats. Dose-effect relationship following urinary excretion of porphyrins. Toxicol. Appl. Pharmacol. 57:156-163.

Cantoni, L., M. Rizzardini, G. Belvedere, R. Cantoni, R. Fanelli, and M. Salmona. 1981a. Induction of mixed-function oxidase by chronic treatment with 2,3,7,8-tetrachloro-dibenzo-p-dioxin in female rats. Toxicology 21:159-167.

Coccia, P., T. Croci, and L. Manara. 1981. Less TCDD persists in liver 2 weeks after single dose to mice fed chow with added charcoal or cholic acid. Br. J. Pharmacol. 72: 181-182.

Doss, M. 1970. Analytical and preparative thin-layer chomatography of porphyrin methyl esters. J. Clin. Chem., Clin., Biochem. 8: 197-207.

Elder, G. H. 1978. Porphyria caused by hexachlorobenzene and other polyhalogenated aromatic hydrocarbons. Pp. 157-200 in Handbook of Experimental Pharmacology, Vol. 44, Heme and Hemoproteins. Springer Verlag, Berlin.

Fanelli, R., M. P. Bertoni, M. Bonfanti, M. G. Castelli, C. Chiabrando, G. P. Martelli, M. A. Noe, A. Noseda, and C. Sbarra. 1980. Routine analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin by gas chromatography-mass fragmentography in biological samples from the contaminated area of Seveso. Bull. Environ. Contam. Toxicol. 24: 818-823.

Goldstein, J. A., P. Hickman, H. Bergman, and J. G. Vos. 1973. Hepatic porphyria induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the mouse. Res. Commun. Chem. Pathol. Pharmacol. 6:919-928.

Greenlee, W. F., and A. Poland. 1978. An improved assay of 7-ethoxycoumarin O-deethylase activity: Induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Pharmacol. Exp. Ther. 205:596-605.

Gupta, B. N., J. G. Vos, J. A. Moore, J. G. Zinkl, and B. C. Bullock. 1973. Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspect. 5:125-140.

Jones, K. G., and G. D. Sweeney. 1977. Association between induction of aryl hydrocarbon hydroxylase and depression of uroporphyrinogen decarboxylase activity. Res. Commun. Chem. Pathol. Pharmacol. 17: 631-637.

Jones, K. G., and G. D. Sweeney. 1980. Dependence of the porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo(p)dioxin upon inheritance of aryl hydrocarbon hydroxylase responsiveness. Toxicol. Appl. Pharmacol. 53:42-49.

Kouri, R. E., H. Ratrie, S. A. Atlas, A. Niwa, and D. W. Nebert. 1974. Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sci. 15:1585-1595.

Lucier, G. W., O. S. McDaniel, G. E. R. Hook, B. A. Fowler, B. R. Sonawane, and E. Faeder. 1973. TCDD-induced changes in rat liver microsomal enzymes. Environ. Health Perspect. 5:199-209.

Mantovani, A., A. Vecchi, W. Luini, M. Sironi, G. P. Candiani, F. Spreafico, and S. Garattini. 1980. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on macrophage and natural killer cell-mediated cytotoxicity in mice. Biomedicine. 32: 200-204.

McConnell, E. E., J. A. Moore, J. K. Haseman, and M. W. Harris. 1977. The comparative toxicity of chlorinated dibenzo-p-dioxin isomers in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44: 335-356.

McConnell, E. E., J. A. Moore, and D. W. Dalgard. 1978. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rhesus monkeys (Macaca mulatta) following a single oral dose. Toxicol. Appl. Pharmacol. 43:175-187.

Nebert, D. W., and H. V. Gelboin. 1968. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I: Assay and properties of induced enzyme. J. Biol. Chem. 243:6242-6249.

Omura, T., and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. I: Evidence for its hemoprotein nature. J. Biol. Chem. 239:2370-2378.

Poland, A., and E. Glover. 1980. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Segregation of toxicity with the Ah locus. Mol. Pharmacol. 17: 86-94.

Poland, A. P., D. Smith, G. Metter, and P. Possick. 1971. A health survey of workers in a 2,4-D and 2,4,5-T plant. With special attention to chloracne, porphyria cutanea tarda, and psychologic parameters. Arch. Environ. Health 22:316-327.

Poland, A. P., E. Glover, J. R. Robinson, and D. W. Nebert. 1974. Genetic expression of aryl hydrocarbon hydroxylase activity. Induction of mono-oxygenase activities and cytochrome P-450 formation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice genetically "nonresponsive" to other aromatic hydrocarbons. J. Biol. Chem. 249:5599-5606.

Robinson, J. R., N. Considine, and D. W. Nebert. 1974. Genetic expression of aryl hydrocarbon hydroxylase induction: evidence for the involvement of other genetic loci. J. Biol. Chem. 249:5851-5859.

Rose, J. Q., J. C. Ramsey, T. H. Wentzler, R. A. Hummel, and P. J. Gehring. 1976. The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36:209-226.

Vecchi, A., A. Mantovani, M. Sironi, W. Luini, M. Cairo, and S. Garattini. 1980. Effect of acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on humoral antibody production in mice. Chem. Biol. Interact. 30:337-342.

Vos, J. G., and J. A. Moore. 1974. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int. Arch. Allergy Appl. Immunol. 47:777-794.

Vos, J. G., J. A. Moore, and J. G. Zinkl. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ. Health Perspect. 5:149-162.

DISCUSSION

DR. MOORE: Over time, there was a marked difference in the compartmentalization of TCDD within the cell. Have you tried to study the charcoal depletion phenomenon, perhaps 14 days after you administered TCDD, when it has left the cytosol and pretty much gone to the microsomal or the mitochondrial fractions? Did you see if, indeed, you could still move it out then?

DR. GARATTINI: Yes. I don't have any results yet, but we are trying to understand if there is a redistribution because of this treatment.

The mechanism is probably dual: one, to drain TCDD from the bile, and two (perhaps more important), to bind the TCDD that is excreted.

DR. WEINBERG: Did you determine exactly what the immediate cause of death was for these animals? Obviously, there is a large toxic effect on the liver and other organs. But were animals dying from toxicity alone? Or was death due to respiratory paralysis or neurologic destruction? Was autopsy analysis detailed enough to determine if other kinds of tissue destruction had occurred?

DR. GARATTINI: I don't know.

DR. MOORE: Some pathologists have necropsied the animals and studied the histopathology. They described a lesion in rodent liver and the absence of a lesion in guinea pig. Then, when asked the cause of death, they say, "I don't know." The simplest answer is that animals look as if they had wasted away.

DR. GARATTINI: Some pathologists believe that the kind of liver degeneration observed, particularly after high doses, is not compatible with life; but it is difficult to answer the question.

DR. NEAL: Have you looked at LD_{50} determinations in the charcoal-treated C57BL/6 mice to see if toxicity is decreased, even though TCDD is cleared from the liver?

DR. GARATTINI: Yes. There is a decrease in mortality.

DR. NEAL: The hamster, for example, may have 100 times more TCDD than the amount found in the liver of the rat, but still sustain no liver damage. Your observation of variable effects on liver with varying doses suggests something really much more complicated.

DR. GARATTINI: Certainly.

DR. NEAL: The wasting syndrome was mentioned, but death can occur from TCDD without this effect. We fed rats parenterally (involuntarily). They gained weight equally with control animals, but they died "right on schedule," without having lost any weight. Weight loss is an indirect effect of the compound.

DR. REHDER: Pathologists are not always able to say how many alterations have been the cause of death, but they can look at a certain histologic picture of the liver and determine whether the alterations were sufficient to cause the death of an animal or a human being. Your histologic pictures showed fatty metamorphosis, swelling of liver cells, and single cell necrosis. These changes are more or less reversible. Could irreversible changes, like fibrosis, bile-duct proliferation, or even cirrhosis be expected? In your experiments, did you wait long enough to see if irreversible changes occurred in the liver?

DR. GARATTINI: Not for liver, but we did for other types of toxicity, such as immunodepression. We found that if the dose is low enough mice can recover completely in about 60 days.

DR. MOORE: TCDD-induced thymic atrophy, after a long enough interval, will show repopulation of the cortical area. The severity of liver pathology in some of the rodent species could be interpreted as a contributing cause of death. However, for the Rhesus monkey or the guinea pig, there is no pathology in the liver.

DR. MURPHY: In your three strains of mice, the C57BL/6 strain was the one susceptible both to toxicity amd thymus atrophy.

The thymus was about twice the weight of that gland in the DBA_2 strain, which was resistant. Is there any connection?

DR. GARATTINI: There are marked differences in the thymus weight, depending on strain. I don't think it has any significance; percentage-wise, there was no decrease in the thymus weight in the DBA2, even with doses that were several times the amount effective in the sensitive strain.

These strains were actually selected on the basis of the effect of TCDD on P-448 induction. The strain that is not sensitive to TCDD, or that is less sensitive to TCDD in terms of liver induction, is also less sensitive in terms of thymus atrophy and also from the point of view of antibody production.

I don't know if the three things are linked. What may be important for this kind of toxicity is a different sensitivity of whatever is the receptor.

In Vivo DNA Damaging Activity, In Vivo Covalent DNA Binding and Bacterial Mutagenicity as Related Quantitatively to Carcinogenic Potency

S. Parodi, M. Taningher, and L. Santi[1]

The following is a brief report on two different studies: one on 16 hydrazine derivatives, and another on 21 compounds in different chemical classes.

For these compounds, the following parameters were examined:

o DNA-damaging potency (DFI). This was evaluated according to the *in vivo* alkaline elution assay, using the following formula: DFI = (K_t - K_c)/(dosage administered in mMol/kg), where K is the elution rate constant of eluted DNA (as a first approximation a parameter directly proportional to the amount of damage).

o Covalent binding index (CBI) according to the formula: CBI = (μmol chemical bound per Mol nucleotides)/(mMol chemical administered/kg).

o Mutagenic potency (MPI) in the Ames test, evaluated according to the following formula: MPI = (hystidine revertants over controls per plate/(nMol of chemical per plate).

o Carcinogenic potency (OPI) evaluated as follows: OPI =-ln (fraction of tumor-free animals)/[(cumulative dose in mMol/kg) x (time of exposure)]. For the set of hydrazine derivatives, OPI was calculated using a slightly different formula.

---

[1] Istituto Scientifico per lo Studio e la Cura dei Tumori and University Department of Oncology, Viale Benedetto XV, 10 16132 Genoa.

The results of these two experiments were the following: (1) The Ames' test was absolutely not predictive for the 16 hydrazine derivatives. (2) The in vivo DNA damage was predictive for the 16 hydrazine derivatives ($r = 0.6$). (3) For the 21 compounds of different chemical classes, all three tests were predictive in the following order: CBI: $r = 0.60$; DFI: $r = 0.55$; MPI: $r = 0.42$.

The correlation between MPI and DFI was very modest ($r = 0.15$). The correlation between CBI and DFI was very strong ($r = 0.80$). A multiple correlation of OPI = f (DFI, MPI) showed improvement in respect to the simple correlations ($r_{123} = 0.64$ against $r_{12} = 0.55$ and $r_{13} = 0.42$). This seems to suggest that a battery of two short-term tests can indeed be more predictive than each one of them individually.

Liver Injury

Sheldon D. Murphy[1]

The liver is a common site of injury resulting from exposure to a wide variety of chemical contaminants. Altered hepatic function and structure often serve as early indicators of excessive exposure. However, because of the liver's large reserve and capacity for repair, chemically induced effects in the liver often do not represent the most serious health effects produced by a particular chemical. It is almost axiomatic that mammals exposed to halogenated organics will have functional and morphologic changes in liver. The nature of these changes and their importance in relation to other effects can differ, depending on the specific compound, the species exposed, the duration of exposure, and the dosage. So many substances induce hepatotoxicity that liver function tests are not very specific early indices of health effects associated with environmental exposure. More insight into the mechanisms of hepatoxicity (in relation to other toxic manifestations) will permit a better assessment of the utility of these tests in epidemiologic followups of chemical contamination.

> *For the king of Babylon stood at the parting of the way, at the head of the two ways, to use divination: he made his arrows bright, he consulted with images, he looked in the liver.--Ezekiel 21:21*

The basis of the following discussion of hepatotoxicity was drawn largely from Zimmerman (1978). The three hepatotoxic candidates for area-wide contamination selected for discussion in this presentation are polychlorinated biphenyls (PCB's), tetrachlorodibenzodioxin (TCDD), and hexachlorobenzene (HCB).

The response of the liver to exposure to hepatotoxic chemicals can take several forms. If the exposure is brief and at a high dosage, and if the chemical is capable of injuring parenchymal cells, acute necrosis can be expected. If injury is sufficiently severe, it can lead to death from hepatic failure in a few days. However, complete recovery can occur, because the liver generally is capable of complete functional and morphologic repair if injury is not too massive or too frequent. A single exposure to chemicals such as white phosphorous can result in massive liver injury, which can lead to macronodular cirrhosis. On the other hand, cirrhosis is more likely to be the outcome of prolonged or frequently repeated exposures that produce repeated subtle injuries. These subtle injuries may never be manifested by characteristic findings in tests of acute liver injury; but in the long term, fibrosis occurs and the chronic clinical condition of cirrhosis becomes manifest.

---

[1] Division of Toxicology, University of Texas Medical School at Houston, Houston, Texas.

At the cellular level, acute exposure to hepatotoxins generally results first in injury to intracellular and cell-surface membranes. With this injury, cellular constituents commonly leak out of the cell and into the extracellular fluids. A measurement of these constituents in plasma is the basis for many of the clinical tests for liver injury. This increased plasma activity of enzymes such as serum glutamic oxaloacetic transaminase (SGOT), and pyruvic transaminase, or isocitric dehydrogenase) has been used as an index of acute hepatocellular injury.

Carbon tetrachloride is often used to illustrate chemical-induced hepatotoxicity. The endoplasmic reticulum of the liver converts carbon tetrachloride to a reactive free radical form. This reactive intermediate actually injures the endoplasmic reticulum and causes decreased activities of enzymes (e.g., glucose-6-phosphatase) associated with that intracellular structure. But this reactive molecule is also capable of reacting with other intracellular and cell-surface membranes. These injurious interactions cause alteration of the critical semipermeable characteristics of the cell, with resultant leakage of constituents into the extracellular fluid. This injury is also manifest by certain structural changes that can be observed by light and electron microscopy. Biochemical manifestations of structural injury can be also detected by assaying for hepatocellular constituents in plasma.

One common outcome of exposure to hepatotoxic chemicals is the accumulation of lipid in the hepatocytes. Free fatty acids are incorporated into triglycerides in the hepatocytes, and the triglycerides are then transported out of the cell. This transport mechanism depends on the presence of very low density lipoproteins. Thus, anything that interferes with the synthesis of such lipoproteins can lead to an impairment of the egress of lipids from the cell and accumulation of triglycerides in the liver (steatosis).

Various compounds that typically cause the accumulation of lipid as one of their manifestations may do so by quite different mechanisms. Dialkyl nitrosamines react with the DNA or RNA that controls the formation of the template for synthesis of the apoprotein that becomes a part of the low density lipoprotein. On the other hand, carbon tetrachloride attacks the membranes of the endoplasmic reticulum and the ribosomes, causing the same net effect--reducing the synthesis of the transport protein. To varying degrees, both aliphatic and aromatic halogenated compounds can produce both necrosis and steatosis.

Table 1 illustrates that a wide variety of aliphatic halogenated hydrocarbons produce both fatty liver and centrizonal necrosis. A few of the compounds seem to be fairly specific in producing only steatosis, and some closely related compounds (some merely stereoisomers of each other) apparently have very little potential for producing liver injury. Although this discussion does not focus on these compounds, consideration of liver injury as a consequence of area-wide chemical contamination probably cannot ignore these relatively low molecular weight, halogenated compounds. Even though these chemicals may not be persistent, many of them are volatile, and they may be ones to which people are exposed through slow vaporization from dumps, leaking storage containers, and so forth.

Table 2 lists several aliphatic and aromatic halogenated compounds that produce hepatic necrosis in laboratory animals. Several of the more persistent of these, such as dichlorodiphenyltrichloroethane (DDT), chloronaphthalenes, and chlorobenzenes, have contributed to area-wide contamination. Some of these chemicals, such as chloroform, are volatile, low molecular weight compounds. Nevertheless, some are fairly ubiquitous at low concentrations in urban potable water supplies because they are formed in the process of water chlorination. Almost all of these compounds, in sufficient doses, can produce centrizonal necrosis of the liver. They are fairly specific in their site of injury within the liver. Most of them will also produce steatosis. As indicated in Table 2, the halogenated compounds are not the only environmental contaminants that produce liver injury.

TABLE 1

Liver Injury by Halogenated Aliphatic Hydrocarbons[a]

Steatosis and Centrizonal Necrosis	Steatosis Only	Slight Steatosis or Injury
CCl_4	CH_2ClBr	CH_3Cl
CI_4	CH_2Cl_2	CH_3Br
CBr_4	$CHCl{=}CHCl$ (cis)	CH_3I
CCl_3Br	$CCl_2{=}CCl_2$	CCl_2F_2
$CHCl_3$	$CH_3CH_2CHClCH_3$	$CHCl{=}CHCl$ (trans)
CHI_3		CH_3CH_2Cl
$CHBr_3$		CH_3CH_2I
$CHCl_2CHCl_2$		CH_3CH_2Br
CH_2ClCH_2Cl		$CH_3CH_2CH_2CH_2Cl$
CH_2BrCH_2Br		
CH_3CCl_3		
$CHCl_2CCl_3$		
$CHCl{=}CCl_2$		
$CH_3CHClCH_3$		
$CH_3CHClCH_2Cl$		

---

[a] From Zimmerman, 1978, with permission.

TABLE 2

Some Halogenated and Nonhalogenated Compounds that Produce Hepatic Necrosis in Experimental Animals[a]

Compounds Halogenated	Site of Necrosis[b]	Accompanied by Steatosis
Bromobenzene	CZ	+
Bromotrichloromethane	CZ	+
Carbon tetrachloride	CZ	+
Chlorobenzenes	CZ	–
Chloroform	CZ	+
Chloroprene	CZ	+
Chlorinated biphenyls	CZ	+
Chloronaphthalenes	CZ	–
Chloropropane	CZ	+
Dichloropropane	CZ	+
Dichlorodiphenyltrichloroethane	CZ	+
Ethylene dibromide	CA	–
Ethylene dichloride	CZ	+
Iodoform	CZ	–
Methylchloroform	CZ	+
Methylene chloride	CZ	+
Nonhalogenated		
Aflatoxins	CZ,PZ	+
Allyl compounds	PZ	–
-Amanitin	CZ	+
Anthrapyrimidine	CZ,MZ	–
Antimony	–	+
Arsenic (inorg.)	CZ,PZ	–
Beryllium	MZ	–
Botulinus toxin	CZ	–
Dimethylnitrosamine	CZ	+
Dinitrobenzene	CZ	+
Ferrous sulfate	PZ	–
Manganese compounds	PZ	–
Naphthalene	CZ	+
Paraquat	CZ,MZ	–
Pyridine	CZ	–
Xylidine	CZ	+

---

[a] Adapted from Zimmerman, 1978.

[b] CZ=centrizonal; MZ=midzonal; PZ=peripheral zonal.

The liver is also a target organ for a variety of other environmental contaminants, not all of which produce centrizonal necrosis. The allyl compounds, for example, act primarily in the portal region. Also, several of the nonhalogenated compounds produce necrosis without characteristically producing fatty livers.

Kuratsune _et al_. (1972) reviewed the signs and symptoms of patients with Yusho disease caused by rice oil contaminated with PCB's. Jaundice was the effect most likely to be associated with liver injury. However, this symptom was reported only at an 11% frequency. Many of the other symptoms occurred much more often. For example, brown pigmentation of the nails was reported at a frequency of 75%-83%. One might suspect that this symptom had some relationship to porphyria, which is often associated with liver injury. Nevertheless, in summarizing the clinical and pathologic findings in Yusho patients, Kuratsune (1972) indicated that there is little objective evidence of liver injury based on standard clinical tests such as plasma enzyme assays. On the other hand, some liver biopsies showed increased endoplasmic reticulum, which is somewhat characteristic of the response observed in laboratory animals exposed to PCB's.

The effects of PCB's in humans, monkeys, and rats are summarized in Table 3. Hepatic hypertrophy is listed as being a response to PCB's exhibited by human and subhuman primates as well as by the rat. But the health significance of hepatic hypertrophy depends on what is causing it, e.g., fatty infiltration, neoplasia, or induced endoplasmic reticulum. Characteristically, PCB's tend to cause increases in liver

TABLE 3

Responses of Primates and Rats to PCB's[a]

Response	Humans	Monkey	Rat
Susceptibility to toxicity	High	High	Moderate
Acne	Yes	Yes	No
Hyperpigmentation of skin	Yes	Infants only	No
Alopecia	NA[b]	Yes	No
Hyperactive meibomian glands	Yes	Yes	No
Conjunctivitis	Yes	Yes	No
Edema of eyelids	Yes	Yes	No
Subcutaneous edema	Yes	Yes	No
Keratin cysts in hair follicles	Yes	Yes	No
Hyperplasia of hair follicle epithelium	Yes	Yes	No
Gastric hyperplasia	NA[b]	Yes	No
Thymic atrophy	NA	NA[b]	Yes
Hepatic hypertrophy	Yes	Yes	Yes
Liver enzyme change	NA[b]	Yes	Yes
Decreased number of red blood cells	Yes	Yes	No
Decreased hemoglobin	Yes	Yes	No
Serum hyperlipidemia	Yes	Hypolipidemia	Yes
Leukocytosis	Yes	Yes	No

---

[a] International Agency for Research on Cancer, 1978, with permission.
[b] NA=not available.

weight and the membranous smooth endoplasmic reticulum. For most of the other toxic symptoms and signs caused by PCB exposure, the rat does not appear to provide a very good model for humans.

Bruckner (1973, 1974) studied rats exposed to Aroclor 1242 and described some of the effects of that compound on the liver. Administering 100 mg/kg of Aroclor 1242 to rats on alternate days for 3 weeks resulted in an approximately two-fold increase in relative liver weight and in SGOT. The increase in SGOT is a minimal effect, since a much greater increase would be expected with serious liver injury. Routinely, some reduction in hematocrit level was also observed. In the rat, the most striking effect of exposure to Aroclor was an approximately tenfold increase in the liver microsomal hydroxylation of acetanilide. However, there was not a uniformly large increase in all microsomal enzyme activities as oxidative demethylase activity increased only a small amount. This liver enzyme-inducing effect persisted in rats exposed to PCB's. After a single dose, the peak induction of hydroxylation activity lasted approximately 5 days. Furthermore, the level of activity remained significantly above that in controls for a longer time, possibly reflecting a flux of PCB's from lipid storage depots into the bloodstream and the liver where the chemical could continue to stimulate the microsomal system. At exposures to concentrations as little as 5 ppm, there was, within a few days, a twofold increase in the activity of acetanilide hydroxylase activity, which persisted for several weeks after the animals were returned to control diets. Additional studies showed that feeding rats Araclor 1242 for 6 months at 5 and 25 ppm in the diet caused a significant increase in liver weight and in liver lipids. There was also a significant twofold increase in the urinary coproporphyrin levels in animals fed as little as 5 ppm. Histologically, the liver had an increased fat content as shown by a Sudan-4 stain. Otherwise, it appeared normal.

Liver damage has been reported to occur in humans exposed to TCDD (National Institute of Environmental Health Sciences, 1978). A review (Kociba et al., 1978) of the results of numerous toxicity studies in laboratory animals indicates that the changes in clinical chemistry associated with liver injury occur at chronic dosage levels of less than 1 mg/kg/day. Thus, it appears that lesions in the liver may be a fairly sensitive index of effect, but apparently no more sensitive than a reduction in fertility in rats (Murray et al., 1979).

Both the thymus and liver are target organs of TCDD. An early study of reported effects of TCDD on relative weights of liver and thymus in guinea pig and mouse indicated that significant changes in the relative weight of liver require a higher dosage than does a change in thymus weight in both species (Harris et al., 1973). This finding could be taken to suggest that thymus is a more sensitive tissue than liver in terms of response to TCDD exposure. However, a later report (McConnell et al., 1978) indicates that changes in relative thymus and liver weights were about equally sensitive for detecting an effect, and neither was more sensitive than reduced body weight gain.

Reproduced from
best available copy.

The discovery of a cytoplasmic TCDD receptor, which appears to have binding affinity for chlorinated dioxins that correlates with their toxicity (Poland et al., 1979), suggested that the presence of these receptors in various tissues may correlate with specific organ susceptibility. Studies by Carlstedt-Duke (1979) in the rat showed that the ratios of TCDD receptor concentrations in extrahepatic tissue to the liver concentrations were 1.81, 1.50, 0.90, 0.25, and 0.20 for thymus, lung, kidney, testis, and brain, respectively. Thus, although liver contained an appreciable quantity of receptor protein, the thymus and lung contained more. The high concentration of TCDD-binding protein in thymus is consistent with the sensitivity of this tissue to injury from TCDD. The lung, however, has not generally been considered a specific target tissue. These molecular studies tend to support the position that the liver is not the most TCDD-sensitive tissue. At the same time, the high concentration of cytoplasmic TCDD-binding protein in the lung fails to make a clear case for the causal association of this protein with specific TCDD organ toxicity.

An early report by Goldstein et al. (1973) demonstrated that TCDD increased liver porphyrin levels in mice given four weekly doses of 25 mg/kg. Under those subacute exposure conditions, lower doses of 1 or 5 mg/kg/week increased liver weight significantly. At the lower doses of TCDD, these increases occurred in the absence of statistically significant changes in porphyrins or of aminolevulinic acid (ALA) synthetase activity. The 5 mg/kg dose group had slight pathological changes in liver. On the other hand, in addition to the increase in porphyrins accompanying the higher dose, there was an increase in ALA synthetase activity, increased iron content, marked liver changes, and 50% mortality of the treated group. These observations suggest that liver porphyrin content was not a good early indicator of toxicity in the mouse. However, in chronic exposures of the rat to TCDD, Kociba et al. (1978) reported increased urinary porphyrin excretion to be one of the most sensitive assays of an effect. The porphyrogenic action of TCDD may thus be more prominent in chronic studies than in the rather high-dose, subacute studies. The specific porphyrin derivatives measured and possible species or strain differences also need to be considered when making such comparisons.

Jones and Butler (1974) made some interesting observations on liver histopathology induced by TCDD. Rats given single TCDD doses of 200 ug/kg were sequentially killed and changes in their livers were observed. Necrotic foci in the centrolobular zone were reported 7 days after the animals received the TCDD. At 14 days, clearly dilated sinusoids were surrounded by enlarged parenchymal cells. Multinucleated cells lined up along the trabeculae in the liver and sometimes surrounded the sinusoids. These multinucleated cells, which the authors considered as somewhat characteristic of response to TCDD exposure, sometimes appeared to have as many as 20 nuclei. Jones and Butler concluded that the TCDD affected the plasma membrane, and that the multinucleated giant cells arose from a coalescing of parenchymal cells--secondary to damage to the cell membranes. Other histologic changes observed at longer times included some mild fibrotic changes and a disordered endoplasmic reticulum. Electronmicrographic observations of liver from TCDD-treated rats were described by Norback and Allen (1972) as including a tortuous disarray of the endoplasmic reticulum, sometimes engulfing other organelles and lipid inclusions.

Hexachlorobenzene (HCB) also produces histopathologic changes which, when viewed by electronmicroscopy, include a disordering of the endoplasmic reticulum that is not too different from that resulting from TCDD. Kuiper-Goodman and coworkers (1977) reported the morphologic and biochemical effects of HCB in relation to tissue residues in rats fed various doses in the diet for 15 weeks. The test dosages ranged from 0.5 to 32 mg/kg/day. Even at the lowest dose, HCB accumulated in liver, but approximately 10 times as much accumulated in adipose tissue. In addition, relative liver weights were increased above those of controls at the two highest dosages of 8 and 32 mg/kg/day. These increased liver weights were associated with increased microsomal oxidase activities. There was consistent histologic evidence of mild liver injury at a dosage of 2 mg/kg/day, and some suggestion of an effect even at the lowest dosage (0.5 mg/kg/day). Liver porphyrin concentrations increased in female rats fed 8 and 32 mg/kg/day during the 15-week exposure period. Lower dosages did not cause liver porphyrin levels to increase during feeding, but an occasional animal at the 2- or 0.5 mg/kg/day dose had an increased liver porphyrin level during the 16-week recovery period. These studies indicated that histopathologic examinations were at least as sensitive in detecting effects of HCB on liver as were measurements of the level of liver porphyrin. The results of plasma enzyme assays of sorbitol dehydrogenase were compared with porphyrin concentrations in liver from rats given 8 and 32 mg/kg/day. The extent of leakage of the liver enzyme into plasma and the increase in porphyrins in liver showed good correlation.

Den Tonkelaar *et al*. (1978) observed pigs fed HCB's in dosages of 0.05, 0.5, 5.0, and 50 mg/kg/day for 90 days. There were significant increases in liver, kidney, and thyroid weights for the 5.0 mg/kg group. Histopathologic changes were observed only in liver of animals receiving less than 50 mg/kg/day. Whorls of smooth endoplasmic reticulum were occasionally seen in pigs fed as little as 0.5 mg/kg/day. No changes of any kind were observed in animals that received 0.05 mg/kg/day. At the 0.5 mg/kg dosage, microsomal oxidase activity in liver was increased significantly as was urinary coproporphyrin excretion. The blood level of HCB corresponding to these minimal biochemical and histopathologic changes in pigs given 0.5 mg/kg/day ranged from 0.235 to 0.285 ppm. This closely corresponded with a 0.338 ppm level of HCB in blood reported (Mazzei and Mazzei, 1973) in a case of chronic HCB intoxication in a human with clinical signs of porphyria and liver damage. Furthermore, the daily dosages that produced evidence of liver injury in both the rat and the pig correspond to the estimated dosage (1 to 4 mg/kg/day) associated with symptoms of porphyria that resulted from an incident of mass chronic poisoning of humans with HCB (Cam and Nigogosyan, 1963).

In conclusion, although tests for liver injury may prove especially sensitive for use in epidemiologic followups after area-wide chemical contamination involving compounds such as HCB, compounds such as PCB's and TCDD may injure the liver only at dosages or durations of exposure that are more severe than those causing serious effects in other organ systems. Clinical tests for liver function or structural integrity are not likely to be very discriminating in an epidemiologic study because so many chemicals that can contaminate the environment have some capacity for liver injury. Thus, although, "looking in the liver" has been one way to search for knowledge and guidance since ancient times, this technique cannot be considered the panacea for today's environmental health problems.

REFERENCES

Bruckner, J. V., K. L. Khanna, and H. H. Cornish. 1973. Biological responses of the rat to polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 24:434-448.

Bruckner, J. V., K. L. Khanna, and H. H. Cornish. 1974. Polychlorinated biphenyl-induced alteration of biologic parameters in the rat. Toxicol. Appl. Pharmacol. 28:189-199.

Cam, C., and G. Nigogosyan. 1963. Acquired toxic porphyria cutanea tarda due to hexachlorobenzene: Report of 438 cases caused by this fungicide. J.A.M.A. 183:88-91.

Carlstedt-Duke, J. M. B. 1979. Tissue distribution of the receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Cancer Res. 39:3172-3176.

Den Tonkelaar, E. M., H. G. Verschuuren, J. Bankovska, T. De Vries, R. Krves, and G. J. Van Esch. 1978. Hexachlorobenzene toxicity in pigs. Toxicol. Appl. Pharmacol. 43:137-145.

Goldstein, J. A., P. Hickman, H. Bergman, and J. G. Vos. 1973. Hepatic prophyria induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the mouse. Res. Commun. Chem. Pathol. Pharmacol. 6:919.

Harris, M. W., J. A. Moore, J. G. Vos, and B. M. Gupta. 1973. General biological effects of TCDD in laboratory animals. Environ. Health Perspect. Exp. Issue No. 5:101-109.

International Agency for Research on Cancer. 1978. Evaluations of the Carcinogenic Risk of Chemicals to Humans: Polychlorinated Biphenyls and Polybrominated Biphenyls. Volume 18. World Health Organization, Lyon, France. 140 pp.

Jones, G., and W. A. Butler. 1974. A morphological study of the liver lesion induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. J. Pathol. 112:93-97.

Kociba, R. J., D. G. Keyes, J. E. Beyer, R. M. Carreon, C. E. Wade, R. P. Dittenber, R.P. Kalnins, L. E. Frauson, C. N. Park, S. A. Barnaed, R. A. Hunmel, and C. G. Humiston. 1978. Results of a two year chronic toxicity and oncogenicity study of 2,3,7-tetrachloro-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46:279-303.

Kuiper-Goodman, T., D. L. Grant, C. A. Moodie, G. O. Korsrud, and I. C. Munro. 1977. Subacute toxicity of hexachlorobenzene in the rat. Toxicol. Appl. Pharmacol. 40:529-549.

Kuratsune, M., T. Yoshimura, J. Matsunaka, and A. Yamaguchi. 1972. Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ. Health Perspect. Exp. Issue No. 1:119-128.

Mazzei, E. S., and C. M. Mazzei. 1973. Une intoxication par un fungicide, l'hexachlorobenzene, souillant les grains de ble. Sem. Hop. Paris. 49:63-69.

McConnell, E. E., J. A. Moore, J. K. Haseman, and M. W. Harris. 1978. The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44:335-356.

Murray, F. J., F. A. Smith, K. D. Nitschke, C. G. Humiston, R. J. Kociba, and B. A. Schwetz. 1979. Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50:241-252.

National Institute of Environmental Health Sciences/International Agency for Research on Cancer. June 1978. Long Term Hazards of Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans. Joint working group report, Internal Technical Report 78/001. International Agency for Research on Cancer, Lyon, France.

Norback, D. H., and J. R. Allen. 1972. Chlorinated aromatic hydrocarbon induced modifications of the hepatic endoplasmic reticulum: Concentric membrane arrays. Environ. Health Perspect. Exp. Issue No. 1:137-143.

Poland, A., W. F. Greenlee, and A. S. Kende. 1979. Studies on the mechanism of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann. N.Y. Acad. Sci. 320:214-230.

Zimmerman, H. J. 1978. Hepatotoxicity, The Adverse Effects of Drugs and Other Chemicals on the Liver. 1978. Appleton-Century-Crofts, New York. 597 pp.

DISCUSSION

DR. GOLDBERG: Dr. Murphy mentioned other effects of TCDD that have not been discussed, and I wonder particularly if sensory effects or peripheral neuropathies have been studied in Seveso.

DR. DARDANONI: Most of the data have been collected, but they have not yet been analyzed. It is difficult to correlate exposure to symptoms and objective signs. Among a number of central nervous symptoms, such as fatigue, sleepiness, and headache, only headache has been found to correlate statistically with chloracne in comparison with the nonchloracne group. This finding holds true both in adults and children. As for effects on the peripheral nervous system, recent studies have demonstrated some sort of damage, measured by nerve conduction and myographic aspects, in addition to direct symptoms such as fatigue, paresthesia, and other subjective observations.

Some of these studies have used control groups rather far from the polluted area and statistically significant differences were found. The method of data collection on subjective symptoms is not entirely free of suspicion of bias. Objective data for the myographic and conduction studies are also under review by several experts.

DR. LINGAMEN: Could some of the neurologic and perhaps some of the psychiatric symptoms be due to porphyria? Acute intermittent porphyria, which is probably a different disease, is associated with psychiatric and neurologic symptoms. Dioxin patients develop porphyria cutanea tarda, and there may be some overlapping of these porphyric syndromes.

DR. NEAL: The porphyria cutanea tarda observed periodically has not been seen consistently. Thus, it is difficult to know whether porphyria cutanea tarda is a symptom of TCDD contamination in humans.

DR. MURPHY: In Turkey, perhaps 15 years ago, a lot of porphyria cutanea tarda resulted. I don't recall any outstanding psychiatric problems associated with that incident.

DR. GOLDBERG: I don't specifically know that incident, but in ongoing studies with lead poisoning, investigators are asking that same question: Are the CNS symptoms associated with lead poisoning due to porphyria? The answers are still very early in development.

Panel on Epidemiologic Approaches to Measurement and Assessment of Exposures

The following Panel was convened to consider the information presented in the Workshop and to extract, if possible, some general principles of investigation of human health impact from extensive environmental contamination:

Prof. P. Bruzzi
Prof. Luigi Dardanoni
Dr. Clark Heath
Dr. George Hutchison
Prof. Cesare Maltoni
Dr. Robert Miller, Moderator
Prof. Bruno Paccagnella

DR. MILLER: In the last minutes of the workshop, we need to develop some ideas about how to organize future studies of area-wide contamination. What action should be taken first after a catastrophe, whether it is radiation contamination, a runaway reaction in a factory, or poisoning of a river?

DR. HUTCHISON: Your question is not how to handle the emergency involving sick people--whether to take them to a hospital or how to treat them--but how to approach the situation. It is generally very difficult to get any cool and objective investigation underway if the population has already realized that something "bad" has happened, and that some "bad" person is probably responsible for it. An early step is to develop some rapport with the people in the area to be studied so that they accept the investigational team. The issues of dealing with the population, with government representatives at all levels, and with commercial interests are tremendously complicated.

After rapport with the public has been established, the investigation should follow the usual epidemiologic steps. Characterize the population, determine the kind of contaminant and estimate exposure to it by area and by individual. Then correlate the data on the population and the outcome with the exposure, over the short and long term.

DR. MILLER: At a series of meetings about 10 years ago, the Defense Atomic Support Agency wanted to know what actions to take if there were a nuclear disaster. A psychiatrist who specialized in disaster studies suggested that the best public relations activity, which was also useful scientifically, was to take a census. Not only is a census valuable for knowing who was exposed, it also gives the people a sense of belonging to society again. It is important to register the population and to get identifying information of a unique sort for each person. In the United States, most adults have a social security number that could be used as an identifier. This would simplify followup studies on exposed population, no matter where an individual goes.

DR. BRUZZI: People can be traced almost everywhere in Italy. The demographic register in municipalities gives forwarding addresses within the area so you can follow people even when they move. The place of residence on the register sometimes does not correspond to the real residence, but on the average it is a good estimator of the population living in each municipality.

An actual census, however, is important too. In Seveso, in August 1979, there was a census of Zone A and of some part of Zone B, but none for Zone R. Nothing was known about the population of the surrounding zones, in which some exposure also occurred. So in August 1979 a census was taken--of the population present now and of a cohort present at the time of exposure.

DR. MILLER: Then a census must count the current population and also try to estimate who was there at the time of the accident and has since left. What percentage of the people had left Seveso?

DR. BRUZZI: We still don't know because we did not match the two populations. We estimate that about 5% of the population is leaving each year. However, the percentage that emigrates is about equal to that which comes in.

DR. HEATH: A census could not have been taken at Three Mile Island right away. We had to wait for the people to return. In the 5-mile radius of the census, maybe 40% of the people had left at some time during the 2 weeks after the accident.

We had to undertake the census quickly, as soon as people came back because, at least in United States, there is a great deal of mobility. In the 2 or 3 months that intervened between the accident and the census at Three Mile Island, many people moved away, not just because of the accident, but because of normal migration.

DR. MILLER: Did the actual mechanism, the procedure of taking a census, have any effect on the population? Did it make the people feel better?

DR. HEATH: I suppose it did, particularly because we recruited census takers from the population itself. The U.S. Census Bureau helped us take the survey, but people from the local community, under the supervision of state and federal personnel, were recruited to interview residents.

DR. MILLER: Identifying the chemical involved in the exposure can take time.

DR. PACCAGNELLA: Certainly, in any area-wide contamination, a first step is to measure the level of contamination. Sometimes it takes a long time, not only because the contaminant is not known, but because there are methodological and analytic aspects to develop and people expert in both analysis and methodology must be gathered. This process takes a long time, sometimes weeks.

Defining the limits of a polluted area and determining the levels of pollution, can take several weeks, as it did in one case when well water was polluted by organic chemicals. In such situations, there is a need to estimate exposure as closely as possible at the beginning, while simultaneously dealing with emotional problems within the population.

DR. MILLER: You have to know what chemical polluted the area and also its source--such as the well water or the cooking oil. For instance, in Japan, is it correct to say that cooking oil was polluted with polychlorinated biphenyls (PCB's)? Wasn't it more than just PCB's? You can be looking for effects from PCB's, but the effects observed may not be due to PCB's if impurities are present. The suspect material must be fully analyzed to know what chemicals are involved.

You need specimens of the chemical to analyze in order to learn what people were actually exposed to. It may not be what you think. Impurities may be more responsible than the compound for whatever effects are observed. So to conduct a proper study, you need to obtain samples of what actually contaminated the area.

DR. HEATH: Sometimes you have to do the best you can, as in the Michigan polybrominated biphenyl (PBB) problem. The contamination happened months before; to retrieve what actually went into the mills and got mixed up in the feed was always a bit uncertain. Even today there is concern that the batch of PBB available for analysis may not have been representative of contamination. Contamination by furans may have occurred and never fully analyzed. Nor can the analysis be made at this date.

Perhaps the most difficult situations are those where people are exposed to multiple chemicals, as at Love Canal or Pittston, Pennsylvania.

DR. PACCAGNELLA: Exposures can be divided into two kinds: those caused by unusual contaminants and those by known contaminants. In the latter situation, criteria and knowledge can be developed in a short time, sometimes even before a disaster. When there is unusual contamination, the situation is quite different and more difficult. Uncertainties and the lack of knowledge impede planning for epidemiologic evaluation and protection.

This lack of certainty and decision making heightens people's emotions. In turn, the ensuing turmoil makes many aspects of organization more difficult.

DR. MALTONI: The source of a calamity may not be known, or if it is known, there may be no data on possible effects to monitor. In Seveso, it was soon known that dioxin was the contaminant, and it was not the first time human beings were exposed to it. Occupational exposures to dioxin had occurred since 1948--in Germany in 1953 at BSF and later at another factory, and in the United Kingdom. These exposures were limited, but strong; yet, in 1976 when the Seveso accident occurred, none of us knew what to do--how to sample, how to monitor, how to behave with respect to a compound with which there had already been experience.

The need emerged, therefore, to have an archive of responses to accidents with known toxic compounds. More than three decades after the first accident in Germany, we do not have epidemiologic data on chronic effects of dioxin.

DR. MILLER: One problem is that analytic methods are still evolving, just as laboratory analyses are. General epidemiologic principles, however, can be applied, whether the exposure is to dioxin, to methylmercury, or to PBB.

There was literature on the subject of accidental exposures, but knowledge of that literature was not instantly available in Milan. You need to know where to go in a particular city to get information quickly on a chemical that you may not even have known was being manufactured in the neighborhood. How can investigators know what they are dealing with? They have to begin by going to the library, unless there is an international or national agency.

DR. MALTONI: In 1976, was public information on the human effects of dioxin sufficiently available on an international basis to develop a behavioral protocol for Seveso?

DR. DARDANONI: There was a delay of 8 days before investigators identified the chemical. It was long known by the factory. Dr. Cavalarro suggested dioxin, because he had studied the literature. The monitoring plan, prepared in a very few weeks, was established on the basis of all data available from the literature. The commission dealing with the Seveso affair stated behavioral norms immediately after the accident. The first people were evacuated very rapidly and the ban on consuming food and animals was established immediately.

DR. MALTONI: I was thinking about the lack of information generally, which could have been provided from knowledge of the previous accident and reports in the international scientific literature. These are two entirely different things. Because so many accidents had happened before, much more should have been known in 1976 at the international level. The scientific community had not collected enough information.

DR. NELSON: Many of these accidents occurred in the privacy of industry and were learned about only later. It was not until 1969 that pieces began to fit together during the Science Advisory Board study of the Agent Orange used in Vietnam. That brought dioxin into consideration of 2,4,5-trichlorophenol. After that, laboratory research accelerated markedly. The earlier episodes were then connected retrospectively, but they were never synthesized. Certainly, by the time of the Seveso accident, there was a vast literature, but it was mostly constructed after the intense interest in Agent Orange. It is a mistake to assume that in 1946 we knew about dioxin. We didn't. It was only in 1969 that the pieces began to fit together.

DR. MOORE: In 1973, a bibliography of all the published scientific data dealing with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was put together. However, although those who worked on it knew of the book's existence, it was not well known to the rest of the world.

Perhaps in this country we profited from an accident like Seveso. We now have available a crisis response team to search literature. Whatever the next area-wide pollutant may be, after it has been identified a complete literature search and printout will be available within 24 hours from the Toxicology Information Response Center (TIRC) at Oak Ridge. The existence of the TIRC, I suspect, as a revelation to the majority of the people in this room. It is under the general auspices of the National Library of Medicine, Department of Health and Human Services.

DR. PACCAGNELLA: There is certainly a need for the literature to be available to everybody. The International Register for Potential Toxic Compounds includes only 10,000-25,000 compounds, in contrast to the 600,000 or 700,000 chemical compounds estimated to be in the environment.

DR. MILLER: Is the Oak Ridge facility open to anybody in the world?

DR. MOORE: Yes, I assume so.

DR. MILLER: After you have taken the census, have identified the exposed people, have identified the chemical, and have the makings of a study, you then have to establish the body burden or the dose. How do you do that?

DR. PACCAGNELLA: It depends on the nature of the chemical. Methylmercury can be measured in blood and in hair. Other organic compounds, such as pesticides, are concentrated in fat.

DR. MILLER: You have to be thinking about what samples to collect, perhaps blood, urine, or fat.

DR. PACCAGNELLA: There is a need for criteria, and sometimes in events of unusual contamination, they are lacking. It is impossible to know in advance what kinds of samples--urine, blood, or hair--to collect. What about biopsies?

DR. MILLER: --And autopsies?

DR. PACCAGNELLA: It is difficult to ask people for a biopsy although it is the correct procedure to follow when you know the kinetics of the compounds. For unusual contaminants, how do you collect biopsies?

DR. BRUZZI: It also depends on the size of the population to be monitored. Blood samples or biopsy specimens are not likely to be obtained from some hundred thousand or ten thousand people.

DR. MILLER: Get material from surgery performed for other reasons and from autopsies. Get specimens from placental or fetal tissue. People may not realize that the placenta is a source of specimens for chemical analysis. Baby teeth and breast milk are other sources. It is important, at the outset of area-wide contamination, to gather specimens appropriate to the chemical for analysis.

DR. PACCAGNELLA: First, you must distinguish between acute or nonacute and usual or unusual contaminants. It is rather difficult to get specimens after acute episodes or unusual contaminations. There may be some other criteria for usual and acute contamination, but the samples mentioned here are certainly important.

DR. HEATH: Among the things to be considered are sampling of the environment and sampling of chemicals both transiently and persistently in the body. Environmental sampling is the other half of the dosimetry question. If an exposure involves large numbers of people, there is no way to collect specimens from all of them. Also, when radiation exposure occurs, dosimetry has to be used to extrapolate the dose from environmental samples. People can't wear film badges all the time in case an accident occurs.

Often sampling is practical only with chemicals that can be measured easily or without too much expense. For chemicals that come one minute and disappear the next, like trichloroethylene, sampling has to take place right at the time of exposure. It is crucial then to know what specimens to get at the time of the accident or soon afterwards. If the contaminants are chemicals that persist for a person's lifetime, specimens can be traced and obtained later. To get specimens from people who die or from aborted tissue at the time of the accident, investigators have to be on top of the situation.

DR. MILLER: How many thousands of years old were the exhumed Peruvian Indians whose bodies provided specimens for lead content?

DR. GOLDBERG: Prehistoric.

DR. MILLER: Samples kept for other purposes can also provide useful information; for instance, in Japan, umbilical cords are traditionally preserved. At Yale University, blood specimens were taken from all entering students. When some students developed infectious mononucleosis, it was easy decades later to pair blood serum to show that Epstein-Barr virus was involved in infectious mononucleosis.

DR. HEATH: To evaluate radiation effects, it has been suggested that old thyroid biopsies or thyroid surgery specimens, saved since days of fallout over southern Utah, be studied.

DR. MILLER: In St. Louis in the 1950's, naturally shed children's teeth were collected to measure fallout. Those teeth are probably retrievable now.

So far now in our hypothetical contamination, the census has been taken, the chemical identified, specimens obtained to analyze the dose, environmental samples taken, the literature reviewed--what next?

DR. HUTCHISON: We haven't identified the effect that we are going to study. Ideally, if the literature is complete, we will know the effects to look for.

Monitoring for acute early effects and long-term effects is totally different. In all likelihood, the long-term effects will be the less well known, and the investigation may turn to identifying etiology, that is, cause-effect relationships. Short-term relationships, in many instances, are already known. But the magnitude of the episode and the persons affected by it must be documented.

DR. MILLER: Let's say we know that people are suffering from severe neurologic disability. How do you find the cases as they develop in a community? Hospitals, practitioners, insurance systems? Death certificates to retrieve information on patients who may have escaped your net?

Look at the system used by the Atomic Bomb Casualty Commission, for example--tissue and tumor registries, hospitals, and patient examinations.

How do you identify patients with specific effects from an exposure? What is being done in Seveso?

DR. BRUZZI: Epidemiologic studies have to be carefully designed. Fishing expeditions have not proved very useful. In Seveso we are establishing two approaches for cancer: first, a mortality study (it is still too soon to get results) and second, an incidence study using a register based on mortality data and hospital records.

Cancer registries, of course, are expensive and difficult to establish. Fortunately, the region is computerizing all admission-discharge information from hospital charts for administrative reasons. The quality of these data is not very good, but it may be useful for getting an idea about the incidence of cancer in the area.

DR. MOORE: It is one thing to collect data to amass a greater base of knowledge for science and mankind, but how do we handle the concerns of the exposed people? A tumor registry isn't really going to help me if I am one of the affected people. People have symptoms that they experience themselves, that they have read about, or somebody has told them might occur. Such symptoms tend to be subjective complaints: "I'm, tired," "My memory isn't as good as it used to be," "My muscles hurt." Or the population might experience a slight increase in fetal resorption, an effect difficult to measure. We aren't doing a very good job of answering the immediate complaints.

DR. HEATH: This is a key problem. Such illnesses--and they are generally the acute ones, the ones that people are worried about immediately--are not going to be found in hospitals. The only approach to take is to examine people and question them about symptoms. It is a very difficult process, and as an epidemiologist I would resist that approach. The medical investigator who has to respond immediately to an exposure cannot say publicly he is not going to give examinations but he might say privately that is his last resort. The trouble with questionnaire surveys and asking people about symptoms is that people perceive their health in relation to what they think their exposure might have been. Distortion occurs. Too often, the findings cannot be documented.

I can't say that in either the Love Canal or Michigan situations the massive physical examinations to find illness possibly related to those exposures have been successful. They have put to rest some of the questions that were raised, but have let others linger. They have opened some difficult situations in Michigan. Perhaps the most difficult concern immunologic problems.

To date, questionnaires have not proved the presence of any abnormality in Michigan or at Love Canal. The one abnormality found at the canal was revealed by using objective records. The investigators had to look at the birth weight on birth certificates; the records showed an apparent increase in the number of children who weighed less than 2,500 g at birth.

DR. MALTONI: The method of monitoring effects is also influenced by the types of diseases sought. One of the most direct and correct methods of cancer followup is epidemiologic evaluation of death certificates. But this takes time, and does not answer people's immediate concerns. For some types of cancer, monitoring the risk is possible; but is not possible for liver cancer. For exposure to vinyl chloride, the use of all possible tests of liver function did not effectively monitor the risk to the population.

Certain procedures, such as sputum cytology, allow for correct monitoring. An example of this is provided by studies of uranium miners in the United States. We have learned to monitor, when possible, for precancerous lesions from the few experiences throughout the world of carcinogenic exposure to chemicals.

Of course, it does not help a person very much to say, "You have such a precursor," because we may not know what to do about it. But we may help science by comparing two populations, exposed and not exposed. Several years later, a mass survey by cytology of sputum or urine will show if an excess of dysplasia exists in the exposed group. This test provides some information about the risk of the exposed group. After experimental data showed that dioxin produced lung tumors in highly resistant animals, such as the rat, the time had come for cytologic monitoring of lung and urine.

DR. MOORE: We need to admit that we do not always do epidemiologic studies well. These studies raise the population's expectations. If we can't deliver, the outome is sometimes worse than doing nothing at all. We should identify those methods that are imprecise and work to improve them. For example, we need better methods to assess a slight decrement in reproductive performance in females in Wyoming. The most helpful records may be those we collect historically, such as birth certificates.

DR. MILLER: Who should respond in this country to area-wide contamination?

DR. HEATH: Area-wide problems are essentially local problems, and the immediate and long-term responsibility rests with local health departments. If the situation is serious enough, it draws in larger bodies such as public health groups and private groups. If your question is who in the Federal group should have that responsibility for the government, the answer is debatable.

DR. MILLER: What happened in the investigation of asbestos exposure in Globe, Arizona.

DR. HEATH: The local people quickly went from step A to step B to step C, and then the Federal people came in.

DR. PACCAGNELLA: In Italy, it is more or less the same. The local authority first has full responsibility. But the public health officers in the villages are really responsible for general public health matters, not for special episodes. They have no technical equipment. They have no means to control environment, to control pollution, to do the analysis. From the legal point of view, however, they are responsible first.

DR. HEATH: Local authorities need to know at what point they should call for assistance from the next higher echelon, and, in the United States, the next step up is the state. Local groups should not hesitate to call for help. A cooperative effort should develop among all levels, but the local one has the continuing responsibility. It is there after the State and Federal Governments go home.

DR. MALTONI: The greatest responsibility lies not with the official there when an accident happens, but with the person (or group) who originally allowed the danger, especially if that official did not assess the danger. In Seveso, the public health officer there when the factory was established was not the one there when the accident occurred. It was known, by the way, that Seveso was the third choice for the factory. Two other places refused to have it.

Under a new law, the mayor of a town is responsible for everything. Under the previous law, both the mayor and the public health officer were equally responsible.

DR. PACCAGNELLA: Yes, but the final decisions are not in the hands of the health authorities. Even before, the final decisions were in the hands of the community through their representatives. If the health authority could limit or stop development, the official would be too powerful. This is true everywhere.

DR. BRUZZI: Cooperation with local services is very important. One of the main failures in Seveso was a break between local health services and the special bureau. The population lost faith in the special bureau because there was no collaboration, physicians were ignored, and there was a gap between these two organizations.

DR. MILLER: Should the local health officer do very sophisticated studies?

DR. HEATH: No study of that sort will be made at the local level. There are exposure incidents where sophisticated studies are not needed, and some practical public health control measures are needed.

If you wanted to conduct sophisticated studies of all accidents, you would have to reorder your priorities. Trichloroethylene exposures in this country, for instance, are a dime a dozen; you would have to tell the local EPA people at the State and county levels not to become involved. You would call on a central Federal person or agency to plug the local study into a large protocol. That would be quite a switch in priorities. Problems that need sophisticated study go through a substantial filtering system in the United States until they reach the top. Now, some that are important get suppressed, not intentionally, but often they are not detected early. We should lubricate the system so it works better.

DR. MILLER: How is TIRC at Oak Ridge being advertised? Do local health officers know it exists?

DR. HEATH: It was mostly set up for agencies at the federal level.

DR. MOORE: Since it was established it has never been called on, perhaps because nobody knows it is there or because no one thinks a specific crisis is big enough. It is a chemical crisis response group; it is not concerned with radiation or infectious diseases.

DR. MILLER: The Department of Transportation listed 603 toxic chemical spills reported to it in 1978. What was the response to those spills?

DR. HEATH: A consortium of federal agencies, called the National Response Team, was set up mainly to handle oil spills. Many of these spills were probably oil spills, and were handled through that team in the initial emergency phase. When the Pittston Mine was found draining chemicals into the Susquehanna River, the National Response Team was called to implement emergency measures. The team works with its state counterparts and deals with the immediate environmental protection aspects of a problem, not necessarily the health aspects. It works on cleaning up the oil, and sequestering the chemicals. It has a fairly practical system. The team currently is under EPA, but often works with the Coast Guard because the team focuses on spills in water.

The National Response Team is concerned not only with water. The Crestview train derailment in northern Florida, which caused considerably more toxic exposure of people than did Three Mile Island, was handled by the southeast regional National Response Team. The team also acted at a fire in a warehouse where toxic chemicals had been stored.

MS. CONWAY: The people within EPA who handle oil spills also handle hazardous waste problems. The Resource Recovery and Conservation Act covers hazardous materials in transportation and disposal. There is a so-called "cradle-to-grave" manifest system for hazardous materials.

DR. IREY: When does the TIRC in Oak Ridge act?

DR. MOORE: It answeres queries, such as, "We had a spill of hexaclorobenzene. We know the name of the chemical; we aren't familiar with what it can do or what previous accidents have shown us, or what is known about it." Within a short period, Oak Ridge will produce a computer search printout of all of the pertinent information in its files. This group does not send anyone out to the field.

DR. PACCAGNELLA: During the last few decades when several epidemiologic studies were conducted on usual environmental pollution (air pollution and respirable disease, pesticides in rural areas, human reactions to traffic or community noise) residents and local health services generally cooperated.

But implementing epidemiologic programs in Italy has been difficult in acute and unusual episodes. In emotional situations, the people in charge of the task forces dealing with the problem lack credibility.

Frequently, sometimes successful attempts have been made during the last 10 years to establish credibility. The people's cooperation has been achieved by asking for international cooperation. It is sort of an international validation of our programs. How do you gain the population's credibility in the United States? Do the people believe in your service? Do they oppose it? Do they believe that you are not objective?

DR. HEATH: When officials are perceived as not reacting promptly, they lose credibility. If they are on the scene doing something, even if it is not much, their activity helps maintain credibility. If they can bring the people to understand that not much can be done, that is ideal. In so many situations, there isn't much that can be done, but you must respond to the public's concern.

DR. PACCAGNELLA: Do the people believe in your service?

DR. HEATH: Well, I hope they do, but we don't always succeed. If people realize the lack of activity is for technical reasons, scientific gaps, or whatever, they will understand the delay. But often a delay is perceived as bureaucratic obfuscation. People think officials don't know what they are doing, or are not doing their jobs.

DR. PACCAGNELLA: Does the public think a task force tries to avoid responsibility?

DR. HEATH: It is a matter of communication and rapid response. Effective rapid response can sometimes be achieved by communicating and by staying at your desk. We often do that. If we can deal with a problem on the phone, that is an adequate response; we don't have to do more. But if a letter sits on our desk for months and gets passed back and forth to different people, we lose credibility.

Drs. Miller and Dardanoni thanked the Panel for their perceptive comments and lucid exchange. Both agreed that this novel analysis of past experiences was a substantial contribution to help guide future investigations of area-wide chemical contamination.

Summary and Conclusions

A workshop was held to explore the steps that should be undertaken to investigate the impact on human health of area-wide chemical contamination. Two approaches were used to explore the topic. On the one hand, case studies of known exposures to toxicants were developed in relation to chemical manufacture. Correspondingly, case studies of types of adverse effects (e.g., reproductive injury, cancer, neurologic effects, genotoxicity, biochemical toxicity, and liver injury) were studied to indicate the complexity of the disease characteristics in relation to various causative substances.

Several studies of acute exposures of humans to substances widely distributed in the environment were identified as likely candidates to provide some insight into investigative approaches and principles demonstrated to be particularly beneficial in establishing cause-effect relationships. The case studies included TCDD ("dioxin"), Yusho (PCBs), Kepone, dibromochloropropane, lead, methyl mercury, chlorinated hydrocarbon mixtures at the Love Canal, polybrominated biphenyls, and acute radiation exposure from the atomic bomb. The case studies exemplified a wide diversity of exposures and of adverse effects, emphasized the complexities of obtaining reliable exposure data, and demonstrated a substantial range of success in delineating cause-effect relationships. A strong basic science data base coupled with creative approaches and carefully controlled study conditions were elements essential to reaching definitive conclusions about impacts on human health.

The second approach focused on various adverse effects, some of which were associated with specific organs (e.g., reproductive toxicity) while others were not (e.g., cancer). This approach elucidated some of the more serious difficulties of finding causes for diseases which are relatively common in an environment where exposures to chemical and physical agents are highly diverse and complex. A variety of study characteristics were identified to enhance the value of such experimental undertakings.

Through the use of the case studies, several general principles emerged:

1. An effective and credible rapport must be established between scientists and the public before any epidemiologic investigation can proceed with its conventional approaches.
2. A necessary first step to the study of an exposed population is a comprehensive census of individuals in the geographic area.
3. To the extent feasible, the contaminant(s) should be identified and its (their) concentraton in the media of human exposure should be documented.
4. If the contaminant(s) is known, literature describing its (their) toxic properties should be reviewed to obtain possible pathologic and biochemical indexes that could be useful leads in epidemiologic studies.
5. In the early phases of investigation, the study should include the analysis of body burdens in order to more accurately assess exposure.
6. Throughout the investigation, humanitarian concern for the exposed population should be preeminent and should dictate a sensitivity in dealing with these individuals such that their anxiety about possible adverse health consequences is kept at a minimum.
7. There was agreement that there should be at least one organization given the responsibility worldwide to investigate the health impact

of large scale chemical contamination; however, no existing agencies were identified as the likely candidates for such a function. The complementary roles of local and national authorities were identified as a subject that needed sharper definition in order to enhance the value of such studies.

8. An important element in the success of such epidemiologic studies is credibility of the investigators and the authorities they represent. Factors influencing credibility include rapidity of response, level of activity, and communications about progress and results. A strong sense of public concern was recommended to tailor interactions in the community and to establish the credibility required to conduct a study whose results have a greater probability of being accepted by the public.

Appendix 1*

Leonardo Santi: Epidemiologic Monitoring in an Episode of Environmental Chemical Pollution: Problems and Programs in the Seveso Experience

When area-wide chemical contamination occurs, particular care must be given to the conciliation of emergency activities with long-term epidemiologic monitoring programs. Immediately after an accident, when surveillance and health care programs are to be set in motion as quickly as possible, programs intended to provide useful epidemiologic data over a longer period may be overlooked. Therefore, emergency activities must be fitted into a general epidemiologic plan with mid-term and long-term monitoring activities.

1. *Ad hoc* intervention programs must be coordinated by a team of epidemiologists.

2. *Ad hoc* activities must be performed, in tandem with health operators and services already present in the area, in order to ensure an early involvement of those who will progressively have to take on the management of the long-term program.

An intervention program that does not account for services already operating in the area is likely to face serious problems later when trying to followup monitored populations.

Furthermore, the basic activities essential to many subsequent epidemiologic studies must be started as soon as possible in order to avoid losing information that is difficult to gather later. Of these activities, a demographic register is a necessary tool for operative purposes, as well as for identifying and following up subgroups of the whole population at different levels of exposure.

---

*This paper was not presented at the workshop but was submitted later.

Another problem that has to be taken care of soon after area-wide contamination is the evaluation, on an individual basis, if possible, of the extent of exposure to the chemicals. At Seveso, emergency problems are over, and long-term monitoring programs have been established. The Epidemiology Department of the Istituto Scientifico per lo Studio e la Cura dei Tumori di Genova was asked to prepare a general plan of epidemiologic research for Seveso. Personnel decided to involve the public health services of the area in the monitoring program and established cooperative ties with the Regione Lombardia's Epidemiological Service and with local health agencies. Now, more than 3 years after the accident, it does not appear fruitful to allocate efforts and resources in ambitious but demanding clinical research protocols involving large population groups. Current research is based on three main criteria: feasibility, clinical and/or social relevance, and association with the polluting agent 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

The first problem was to define the evaluation of exposure and, at the same time, to select suitable control groups, which are essential for epidemiologic studies. Because reliable indicators of dioxin exposure are not available (even chloracne, which is a quite specific indicator, does not seem to be very sensitive), and information about individual exposure was scanty and of poor quality, it was decided to use place of residence at the moment of the accident and during the following periods for the stratification of the population into subgroups having received different levels of TCDD exposure. Due to the organizational and administrative impossibility of selecting and following suitable control groups outside the area, it became necessary to identify areas inside the monitored zone that can be regarded as free of TCDD contamination. This judgment was based on data concerning the amount of TCDD in soil, animal casualties, and acute dermal lesions or chloracne in individuals in every area. The population of the TCDD-free areas serves as control groups for the large epidemiologic studies.

The following groups of individuals, however, are classified as exposed, independent of their place of residence:

1. individuals with acute or chronic skin lesions attributable to the toxic cloud or to TCDD;
2. occupationally exposed workers; and
3. individuals in highly polluted zones at the time of the accident.

These groups are to be followed with specific protocols, along with suitable control groups.

Some basic studies were given top priority because they were expected to provide general and essential information and to serve as preliminary steps for more specific research. These included:

1. a mortality study, both on the current population of the area and on the cohort present in July 1976;

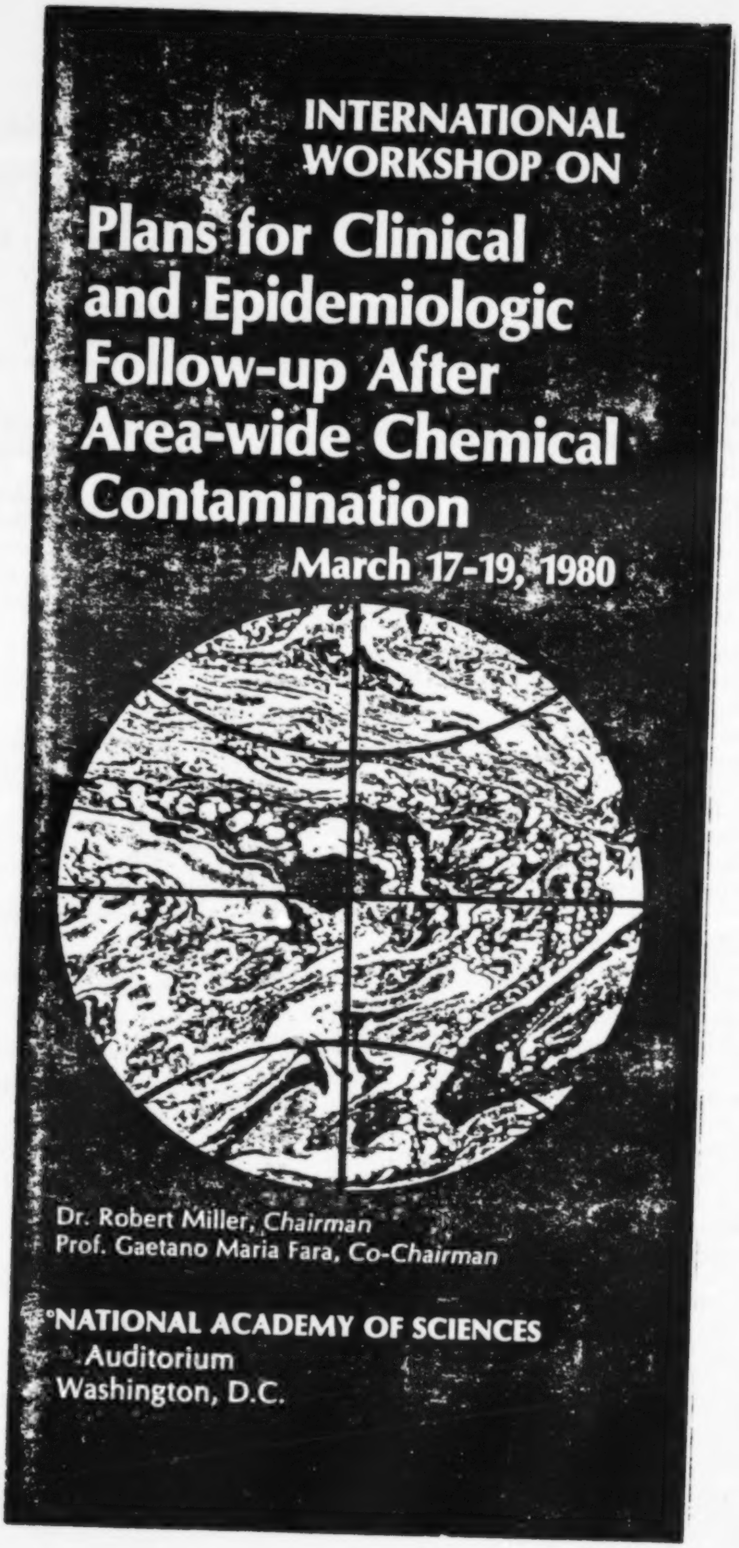
2. a cancer incidence study by means of a cancer registry; and

3. a study of the frequency of birth defects in newborns from 1976 on and of abortions performed in the area.

These programs are being performed now, along with detailed followup protocols for different subgroups at defined levels of exposure.

This program, admittedly smaller than that planned earlier, is still comprehensive and seems feasible in all its parts. It will not provide final answers to all questions raised by the ICMESA accident, but will generate reliable data regarding most of the major problems.

Appendix 2



INTERNATIONAL
WORKSHOP ON

Plans for Clinical and Epidemiologic Follow-up After Area-wide Chemical Contamination

March 17-19, 1980

Dr. Robert Miller, *Chairman*
Prof. Gaetano Maria Fara, *Co-Chairman*

NATIONAL ACADEMY OF SCIENCES
Auditorium
Washington, D.C.

Sponsored by

COMMITTEE ON RESPONSE STRATEGIES
TO UNUSUAL CHEMICAL HAZARDS

BOARD ON TOXICOLOGY AND
ENVIRONMENTAL HEALTH HAZARDS

Assembly of Life Sciences

National Research Council

National Academy of Sciences

PURPOSE

Through the review of various episodes of area-wide contamination by chemical and physical agents, this workshop will seek general principles and formulate plans for the investigation of wide-spread exposures and their impacts on human health.

COMMITTEE ON RESPONSE STRATEGIES TO UNUSUAL CHEMICAL HAZARDS

Dr. Norton Nelson, Chairman, Institute of Environmental Medicine, New York University Medical Center, New York, N.Y. 10016

Dr. A. L. Burlingame, Mass Spectrometry Research Resource, University of California, Berkeley, Calif. 94720

Dr. Aaron B. Lerner, Department of Dermatology, Yale University, New Haven, Conn. 06510

Dr. Robert Miller, Vice-Chairman, Epidemiologic Branch, National Cancer Institute, Bethesda, Md. 20205

Dr. John A. Moore, Research Resources Program, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709

Dr. Sheldon D. Murphy, Department of Pharmacology, University of Texas, Houston, Tx. 77025

Dr. Robert A. Neal, Department of Biochemistry, Vanderbilt University, Nashville, Tenn. 37203

Dr. Milos Novotny, Department of Chemistry, Indiana University, Bloomington, Ind. 47401

Dr. Patrick O'Keefe, New York State Department of Health, Albany, N.Y. 12201

Dr. Alan Poland, Department of Oncology, University of Wisconsin, Madison, Wis. 53706

PROGRAM

MONDAY, MARCH 17, 1980

8:00 REGISTRATION AND COFFEE

9:00 WELCOME
Dr. Philip Handler, *President*
National Academy of Sciences

INTRODUCTION
Dr. Norton Nelson
Prof. Francesco Pocchiari

I. CASE STUDIES OF SELECTED AREA-WIDE ENVIRONMENTAL EXPOSURES

A brief background description of each occurrence will be presented with emphasis on the source(s) of contamination, nature and degree of exposure, population exposed, and other pertinent exposure assessment parameters.

9:30 TCDD (Italy)
Prof. Gaetano Fara

9:45 TCDD (U.S.)
Dr. Raymond Suskind

10:00 Yusho Disease (Japan)
Dr. Robert Miller

10:15 COFFEE

10:30 Kepone (U.S.)
Dr. Philip Guzelian

10:45 DBCP (U.S.)
Dr. Donald Whorton

11:00 Lead (U.S.)
Dr. Philip Landrigan

11:15 Methyl Mercury (Japan)
Dr. Robert Miller

11:30 Chlorinated Hydrocarbons (U.S. Love Canal)
Dr. David Axelrod

12:00 LUNCH

1:30 PBB's (U.S.)
Dr. Philip Landrigan

1:45 ABCC* (Japan)
Dr. Gilbert Beebe

*Atomic Bomb Casualty Commission

II. ADVERSE EFFECTS ON TARGET SITES

REPRODUCTIVE INJURY

2:00 Dr. David Axelrod

2:40 Prof. Gianni Remotti

3:20 COFFEE

3:30 Dr. Robert Miller

4:10 Dr. Helga Rehder

TUESDAY, MARCH 18, 1980

DERMATOLOGIC EFFECTS

9:00 Dr. Aaron Lerner

9:40 Prof. Vittorio Puccinelli

10:20 COFFEE

CLINICAL EFFECTS OF EXPOSURES IN U.S. TO 2,4,5-T AND ITS CONTAMINANTS

10:30 Dr. Raymond Suskind

CARCINOGENIC EFFECTS

11:10 **Human Observations**
Dr. Clark Heath

11:50 **Experimental Studies**
Prof. Guiseppe Della Porta

12:30 LUNCH

1:30 **Experimental Studies**
Dr. John Moore

NEUROLOGICAL AND BEHAVIORAL EFFECTS

2:10 Dr. Alan Goldberg

2:50 Prof. Guglielmo Scarlato

3:30 COFFEE

IMMUNOLOGICAL EFFECTS

3:45 Dr. John Moore

4:25 Prof. Girolamo Sirchia

WEDNESDAY, MARCH 19, 1980

SOMATIC CELL MUTATIONS

9:00 Dr. Arthur Bloom

9:40 Prof. Luigi De Carli

10:20 COFFEE

LIVER INJURY AND OTHER TOXIC EFFECTS

10:30 Dr. David Axelrod

11:10 Prof. Carlo Zanussi

12:00 LUNCH

1:30 Dr. Robert Neal

2:10 Prof. Silvio Garattini

2:50 Dr. Sheldon Murphy

3:30 COFFEE

⇨

ADVANCE REGISTRATION

There is no charge for registration. Registering in advance is required.

The registration form and any inquiries concerning the program should be addressed to:

Dr. Robert G. Tardiff
Executive Director
Board on Toxicology and Environmental
Health Hazards
National Academy of Sciences
2101 Constitution Avenue
Washington, D.C. 20418
(202) 389-6914

Name ____________________

Organization ____________________

Address ____________________

City ____________ *State* ________ *Zip* ________

Telephone ____________________

3:45

III. PANEL ON EPIDEMIOLOGIC APPROACHES TO MEASUREMENT AND ASSESSMENT OF EXPOSURES

Prof. Luigi Dardanoni
Dr. Clark Heath
Dr. George Hutchison
Dr. Robert Miller, Moderator
Prof. Bruno Paccagnella
Prof. Alfredo Zampieri

4:45

IV. SUMMARY AND PROJECTIONS FOR THE FUTURE

Dr. Robert Miller
Prof. Gaetano Fara

GENERAL INFORMATION

Registration and Luncheons

Badges will be issued to all registrants at registration desks at the C Street entrance of the National Academy of Sciences between 8:00 and 9:00 a.m.

Tickets for luncheons will be provided for participants of the workshop on March 17-19 and sold to all others at a cost of $4.50 per day.

Message Center

Incoming calls to meeting attendees should be directed to (202) 389-6821. Messages received will be placed on the bulletin board at the entrance to the NAS Auditorium.

Parking

The Academy does not have parking facilities for participants or attendees. However, if you plan to drive, some side street parking is usually available.

PARTICIPANTS OF THE INTERNATIONAL WORKSHOP ON PLANS FOR CLINICAL AND EPIDEMIOLOGIC FOLLOW-UP AFTER AREA-WIDE CHEMICAL CONTAMINATION

DR. DAVID AXELROD, Commissioner of Health, New York State Department of Health, Nelson A. Rockefeller Empire State Plaza, Albany, N.Y. 12237

DR. GILBERT BEEBE, Clinical Epidemiology Branch, National Cancer Institute, Bethesda, Md. 20205

DR. ARTHUR BLOOM, Department of Pediatrics, Columbia University, New York, N.Y. 10032

PROF. LUIGI DARDANONI, Istituto di Igiene, Universita di Palermo, 90100 Palermo, Italy

PROF. LUIGI DE CARLI, Istituto Genetica, Facolta Scienza, Via S. Epifanio 14, 27100 Pavia, Italy

PROF. GIUSEPPE DELLA PORTA, Istituto per lo Studio dei Tumori, Via Venziani, 20100 Milano, Italy

PROF. GAETANO MARIA FARA, Istituto di Igiene, Universita di Milano, 20100 Milano, Italy

PROF. SILVIO GARATTINI, Istituto Ricerche Farmacologiche "Mario Negri," Via Eritre A 62, 20157 Milano, Italy

DR. ALAN GOLDBERG, Department of Environmental Health, Johns Hopkins University, Baltimore, Md. 21025

DR. PHILIP GUZELIAN, Medical College of Virginia, Box 265, Richmond, Va. 23298

DR. CLARK HEATH, Chronic Diseases Division, Bureau of Epidemiology, Center for Disease Control, Atlanta, Ga. 30333

DR. GEORGE HUTCHISON, Department of Epidemiology, Harvard School of Public Health, Boston, Mass. 02115

DR. PHILIP LANDRIGAN, NIOSH/DSHEFS/MSF-1, Robert Taft Laboratories, Cincinnati, Ohio 45226

DR. AARON LERNER, Department of Dermatology, Yale University, New Haven, Conn. 06510

DR. ROBERT MILLER, Epidemiologic Branch, National Cancer Institute, Bethesda, Md. 20205

DR. JOHN MOORE, Research Resources Program, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709

DR. SHELDON D. MURPHY, Department of Pharmacology, University of Texas, Houston, Tx. 77025

DR. ROBERT NEAL, Department of Biochemistry, Vanderbilt University, Nashville, Tenn. 37203

DR. NORTON NELSON, Institute of Environmental Medicine, New York University Medical Center, 550 First Street, New York, N.Y. 10016

PROF. BRUNO PACCAGNELLA, Istituto di Igiene, Universita di Padova, 35100 Padova, Italy

PROF. FRANCESCO POCCHIARI, Direttore, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy

PROF. VITTORIO PUCCINELLI, Clinica Dermatologica, Universita di Milano, 20100 Milano, Italy

DR. HELGA REHDER, Institut fur Pathologie Medizinischen Hochschule Lubeck, Ratzeburger Allee 160, D-24, Lubeck, Lubeck, West Germany

PROF. GIANNI REMOTTI, Prima Clinica Osterica, Universita di Milano, Via della Commenda 12, 20110 Milano, Italy

PROF. GUGLIELMO SCARLATO, Istituto Neurologia, Universita di Milano, Via. A. Sforza 35, 20100 Milano, Italy

PROF. VITTORIO SILANO, Laboratorio di Tossicologia, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy

PROF. GIROLAMO SIRCHIA, Ospedale Maggiore Policlinco di Milano, Via F. Sforza 35, 20122 Milano, Italy

DR. RAYMOND SUSKIND, Kettering Laboratories, University of Cincinnati, Cincinnati, Ohio 45219

DR. DONALD WHORTON, Labor Occupational Health Program, University of California, Berkeley, Calif. 94720

PROF. ALFREDO ZAMPIERI, Laboratorio di Epidemiologia et Biostatistica, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy

PROF. CARLO ZANUSSI, Clinica Medica. Quarta, Padiglione Litta, Via Sforza 35, 20122 Milano, Italy

NAS NAE IOM NRC National Academy Press

The National Academy Press was created by the National Academy of Sciences to publish the reports issued by the Academy and by the National Academy of Engineering, the Institute of Medicine, and the National Research Council, all operating under the charter granted to the National Academy of Sciences by the Congress of the United States.